

\* \* \* \* \* STN Columbus \* \* \* \* \*

FILE 'HOME' ENTERED AT 15:53:09 ON 16 DEC 2003

=> fil .bec

COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
0.21	0.21

FULL ESTIMATED COST

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS, ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 15:53:51 ON 16 DEC 2003  
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s (humaniz? or synthetic) (8a)gene/q

FILE 'MEDLINE'

2125 HUMANIZ?

129826 SYNTHETIC

L1 7992 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'SCISEARCH'

2095 HUMANIZ?

142489 SYNTHETIC

L2 6519 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'LIFESCI'

630 HUMANIZ?

37104 SYNTHETIC

L3 4264 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'BIOTECHDS'

3091 HUMANIZ?

11713 SYNTHETIC

L4 2925 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'BIOSIS'

2046 HUMANIZ?

187588 SYNTHETIC

L5 8030 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'EMBASE'

1802 HUMANIZ?

99517 SYNTHETIC

L6 6688 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'HCAPLUS'

4123 HUMANIZ?

525491 SYNTHETIC

L7 14701 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'NTIS'

136 HUMANIZ?

18524 SYNTHETIC

L8 165 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'ESBIOBASE'

976 HUMANIZ?

34574 SYNTHETIC

L9 2994 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'BIOTECHNO'

837 HUMANIZ?

41108 SYNTHETIC

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L10      5313 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

FILE 'WPIDS'
      3082 HUMANIZ?
      200405 SYNTHETIC
L11      1861 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

TOTAL FOR ALL FILES
L12      61452 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

=> s l12 and codon preference
FILE 'MEDLINE'
      31897 CODON
      28985 PREFERENCE
      85 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L13      9 L1 AND CODON PREFERENCE

FILE 'SCISEARCH'
      22598 CODON
      38791 PREFERENCE
      79 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L14      6 L2 AND CODON PREFERENCE

FILE 'LIFESCI'
      13273 "CODON"
      15437 "PREFERENCE"
      59 CODON PREFERENCE
          ("CODON" (W) "PREFERENCE")
L15      5 L3 AND CODON PREFERENCE

FILE 'BIOTECHDS'
      4307 CODON
      677 PREFERENCE
      14 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L16      4 L4 AND CODON PREFERENCE

FILE 'BIOSIS'
      26712 CODON
      45623 PREFERENCE
      99 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L17      7 L5 AND CODON PREFERENCE

FILE 'EMBASE'
      25265 "CODON"
      27954 "PREFERENCE"
      64 CODON PREFERENCE
          ("CODON" (W) "PREFERENCE")
L18      7 L6 AND CODON PREFERENCE

FILE 'HCAPLUS'
      31452 CODON
      33617 PREFERENCE
      120 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L19      13 L7 AND CODON PREFERENCE

FILE 'NTIS'
      88 CODON
      2828 PREFERENCE
      1 CODON PREFERENCE

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                (CODON(W) PREFERENCE)
L20              0 L8 AND CODON PREFERENCE

FILE 'ESBIOBASE'
    12730 CODON
    12820 PREFERENCE
    34 CODON PREFERENCE
        (CODON(W) PREFERENCE)
L21              4 L9 AND CODON PREFERENCE

FILE 'BIOTECHNO'
    21901 CODON
    6421 PREFERENCE
    62 CODON PREFERENCE
        (CODON(W) PREFERENCE)
L22              7 L10 AND CODON PREFERENCE

FILE 'WPIDS'
    2187 CODON
    4177 PREFERENCE
    4 CODON PREFERENCE
        (CODON(W) PREFERENCE)
L23              1 L11 AND CODON PREFERENCE

TOTAL FOR ALL FILES
L24              63 L12 AND CODON PREFERENCE

=> s l12 and (transcription(3a) (regulat? or factor#) or splice or promoter# or
poly(w) 'a' or polyadenylat?)
FILE 'MEDLINE'
    208884 TRANSCRIPTION
    644055 REGULAT?
    2045854 FACTOR#
    87022 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
    11474 SPLICE
    102802 PROMOTER#
    51190 POLY
    7349550 'A'
    15264 POLY(W) 'A'
    6451 POLYADENYLAT?
L25              1407 L1 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'SCISEARCH'
    167445 TRANSCRIPTION
    561638 REGULAT?
    1188154 FACTOR#
    71846 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
    12620 SPLICE
    107233 PROMOTER#
    144243 POLY
    9119811 'A'
    7375 POLY(W) 'A'
    5049 POLYADENYLAT?
L26              821 L2 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'LIFESCI'
    89396 TRANSCRIPTION
    224420 REGULAT?
    274624 FACTOR#
    36944 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
    5961 SPLICE
    60074 PROMOTER#

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16551 POLY
1952664 'A'
5816 POLY(W) 'A'
4024 POLYADENYLAT?
L27 702 L3 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'BIOTECHDS'
12671 TRANSCRIPTION
23294 REGULAT?
32011 FACTOR#
2490 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
1042 SPLICE
28993 PROMOTER#
5854 POLY
307037 'A'
1758 POLY(W) 'A'
1410 POLYADENYLAT?
L28 893 L4 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'BIOSIS'
194495 TRANSCRIPTION
713706 REGULAT?
1156108 FACTOR#
71585 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
12428 SPLICE
116247 PROMOTER#
136884 POLY
7451954 'A'
11783 POLY(W) 'A'
7366 POLYADENYLAT?
L29 1087 L5 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'EMBASE'
191474 TRANSCRIPTION
536945 REGULAT?
1039629 FACTOR#
83515 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
10159 SPLICE
86706 PROMOTER#
44205 POLY
6478350 'A'
8354 POLY(W) 'A'
6671 POLYADENYLAT?
L30 875 L6 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'HCAPLUS'
235973 TRANSCRIPTION
797533 REGULAT?
1295241 FACTOR#
122013 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
14284 SPLICE
160759 PROMOTER#
595071 POLY
17535590 'A'
17365 POLY(W) 'A'
10451 POLYADENYLAT?
L31 2815 L7 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'NTIS'

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      1572 TRANSCRIPTION
      81615 REGULAT?
      144850 FACTOR#
      424 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
      454 SPLICE
      1519 PROMOTER#
      5513 POLY
      1635000 'A'
      64 POLY(W) 'A'
      9 POLYADENYLAT?
L32      11 L8 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'ESBIOBASE'
      94311 TRANSCRIPTION
      314159 REGULAT?
      364832 FACTOR#
      46581 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
      7278 SPLICE
      56402 PROMOTER#
      14526 POLY
      1952041 'A'
      3050 POLY(W) 'A'
      2443 POLYADENYLAT?
L33      464 L9 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'BIOTECHNO'
      159702 TRANSCRIPTION
      270192 REGULAT?
      294703 FACTOR#
      64864 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
      8841 SPLICE
      76053 PROMOTER#
      21581 POLY
      1447807 'A'
      6315 POLY(W) 'A'
      5840 POLYADENYLAT?
L34      884 L10 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'WPIDS'
      10730 TRANSCRIPTION
      346671 REGULAT?
      139224 FACTOR#
      2494 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
      9057 SPLICE
      30677 PROMOTER#
      149681 POLY
      1462261 'A'
      200 POLY(W) 'A'
      762 POLYADENYLAT?
L35      402 L11 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

TOTAL FOR ALL FILES
L36      10361 L12 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
      PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

=> s l36 and (increas? or high) (2a)express? and muta?
FILE 'MEDLINE'
      1796738 INCREAS?
      1164532 HIGH
      794409 EXPRESS?

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62766 (INCREAS? OR HIGH) (2A)EXPRESS?  
 424085 MUTA?  
 L37 32 L25 AND (INCREAS? OR HIGH) (2A)EXPRESS? AND MUTA?  
 FILE 'SCISEARCH'  
 1781642 INCREAS?  
 1700209 HIGH  
 1015504 EXPRESS?  
 61830 (INCREAS? OR HIGH) (2A)EXPRESS?  
 406348 MUTA?  
 L38 23 L26 AND (INCREAS? OR HIGH) (2A)EXPRESS? AND MUTA?  
 FILE 'LIFESCI'  
 461803 INCREAS?  
 323327 HIGH  
 331143 EXPRESS?  
 24465 (INCREAS? OR HIGH) (2A)EXPRESS?  
 191874 MUTA?  
 L39 15 L27 AND (INCREAS? OR HIGH) (2A)EXPRESS? AND MUTA?  
 FILE 'BIOTECHDS'  
 57173 INCREAS?  
 60586 HIGH  
 104880 EXPRESS?  
 5662 (INCREAS? OR HIGH) (2A)EXPRESS?  
 36407 MUTA?  
 L40 25 L28 AND (INCREAS? OR HIGH) (2A)EXPRESS? AND MUTA?  
 FILE 'BIOSIS'  
 1954247 INCREAS?  
 1308070 HIGH  
 976647 EXPRESS?  
 66119 (INCREAS? OR HIGH) (2A)EXPRESS?  
 476365 MUTA?  
 L41 27 L29 AND (INCREAS? OR HIGH) (2A)EXPRESS? AND MUTA?  
 FILE 'EMBASE'  
 1697357 INCREAS?  
 1129744 HIGH  
 725189 EXPRESS?  
 57747 (INCREAS? OR HIGH) (2A)EXPRESS?  
 349714 MUTA?  
 L42 16 L30 AND (INCREAS? OR HIGH) (2A)EXPRESS? AND MUTA?  
 FILE 'HCAPLUS'  
 3576345 INCREAS?  
 3278698 HIGH  
 981208 EXPRESS?  
 64248 (INCREAS? OR HIGH) (2A)EXPRESS?  
 435995 MUTA?  
 L43 42 L31 AND (INCREAS? OR HIGH) (2A)EXPRESS? AND MUTA?  
 FILE 'NTIS'  
 178606 INCREAS?  
 317413 HIGH  
 36920 EXPRESS?  
 556 (INCREAS? OR HIGH) (2A)EXPRESS?  
 9497 MUTA?  
 L44 0 L32 AND (INCREAS? OR HIGH) (2A)EXPRESS? AND MUTA?  
 FILE 'ESBIOBASE'  
 606429 INCREAS?  
 389803 HIGH  
 430586 EXPRESS?

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38456 (INCREAS? OR HIGH) (2A) EXPRESS?
203425 MUTA?
L45      11 L33 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

FILE 'BIOTECHNO'
381709 INCREAS?
297809 HIGH
449467 EXPRESS?
38457 (INCREAS? OR HIGH) (2A) EXPRESS?
241118 MUTA?
L46      16 L34 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

FILE 'WPIDS'
1140528 INCREAS?
1769077 HIGH
95457 EXPRESS?
3718 (INCREAS? OR HIGH) (2A) EXPRESS?
23148 MUTA?
L47      11 L35 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

TOTAL FOR ALL FILES
L48      218 L36 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

=> s l24 or l48
FILE 'MEDLINE'
L49      41 L13 OR L37

FILE 'SCISEARCH'
L50      29 L14 OR L38

FILE 'LIFESCI'
L51      20 L15 OR L39

FILE 'BIOTECHDS'
L52      29 L16 OR L40

FILE 'BIOSIS'
L53      34 L17 OR L41

FILE 'EMBASE'
L54      23 L18 OR L42

FILE 'HCAPLUS'
L55      55 L19 OR L43

FILE 'NTIS'
L56      0 L20 OR L44

FILE 'ESBIOBASE'
L57      15 L21 OR L45

FILE 'BIOTECHNO'
L58      23 L22 OR L46

FILE 'WPIDS'
L59      12 L23 OR L47

TOTAL FOR ALL FILES
L60      281 L24 OR L48

=> dup rem l60
PROCESSING COMPLETED FOR L60
L61      111 DUP REM L60 (170 DUPLICATES REMOVED)

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=> d tot 1-10

L61 ANSWER 1 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI New recombinant nucleic acid, useful for assessing a **mutagenic**  
potential of a test agent in a tissue culture or a transgenic non-human  
animal;

recombinant enzyme protein production via plasmid expression in host  
cell useful for assessing a **mutagenic** potential of a test  
agent

AU SHORT J M; SWANSON R V; BYLINA E; MATHUR E J; LAM D E  
AN 2003-24497 BIOTECHDS  
PI WO 2003068910 21 Aug 2003

L61 ANSWER 2 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI New high density output array of multiple yeast strains, useful for  
functional analysis of genetic and protein-protein interactions to screen  
for function of biological effectors on yeast;

DNA array for proteomics, gene function and protein-protein  
interaction study

AU BOONE C  
AN 2003-11319 BIOTECHDS  
PI WO 2003016568 27 Feb 2003

L61 ANSWER 3 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
TI Efficient protein expression systems using vectors containing  
**mutated T5 promoter/operator** regulatory sequences

SO PCT Int. Appl., 47 pp.  
CODEN: PIXXD2

IN Hu, Mary Chaohong  
AN 2003:571164 HCAPLUS  
DN 139:129102

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003060143	A2	20030724	WO 2002-US34768	20021028
WO 2003060143	A3	20031113		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MY, NZ, OM, PH,  
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,  
UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD,  
RU, TJ, TM  
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,  
CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,  
PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,  
NE, SN, TD, TG

US 2003143685	A1	20030731	US 2002-284083	20021028
US 2003143245	A1	20030731	US 2002-284400	20021028

L61 ANSWER 4 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
TI Sequences of a mouse G protein-coupled receptor P2T, and diagnostic and  
therapeutic use

SO PCT Int. Appl., 166 pp.  
CODEN: PIXXD2

IN Pausch, Mark Henry  
AN 2003:173474 HCAPLUS  
DN 138:216556

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003018066	A2	20030306	WO 2002-US26005	20020815
WO 2003018066	A3	20030522		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,

LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,  
 PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,  
 UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,  
 CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,  
 PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,  
 NE, SN, TD, TG

- L61 ANSWER 5 OF 111 MEDLINE on STN  
 TI Two CCAAT/enhancer binding protein sites in the cytochrome P4503A1 locus.  
 Potential role in the glucocorticoid response.  
 SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (2003 Feb) 270 (3) 556-64.  
 Journal code: 0107600. ISSN: 0014-2956.  
 AU Rodrigues Elsa; Vilarem Marie-Jose; Ribeiro Vera; Maurel Patrick; Lechner  
 Maria C  
 AN 2003036356 MEDLINE
- L61 ANSWER 6 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
 TI Targeted introduction of a diphtheria toxin resistant **mutation**  
 into the chromosomal EF-2 locus of *Pichia pastoris* and expression of  
 immunotoxin in the EF-2 **mutants**  
 SO Protein Expression and Purification (2003), 30(2), 262-274  
 CODEN: PEXPEJ; ISSN: 1046-5928  
 AU Liu, Yuan Yi; Woo, Jung Hee; Neville, David M.  
 AN 2003:563999 HCAPLUS  
 DN 139:286968
- L61 ANSWER 7 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
 TI High-level **expression** of hemoglobin A in human  
 thalassemic erythroid progenitor cells following lentiviral vector  
 delivery of an antisense snRNA  
 SO Blood (2003), 101(1), 104-111  
 CODEN: BLOOAW; ISSN: 0006-4971  
 AU Vacek, Maria M.; Ma, Hong; Gemignani, Federica; Lacerra, Giuseppina;  
 Kafri, Tal; Kole, Ryszard  
 AN 2003:11100 HCAPLUS  
 DN 139:680
- L61 ANSWER 8 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
 TI New recombinant polypeptide with cellulase activity, useful for  
 identifying cellulase substrate or modulator of cellulase activity, for  
 making small molecule, and for treating printed paper;  
 recombinant enzyme protein production for use in small molecule  
 identification  
 AU SHORT J M; MATHUR E J; LAM D E  
 AN 2003-09417 BIOTECHDS  
 PI WO 2002101078 19 Dec 2002
- L61 ANSWER 9 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
 TI Novel inducible growth hormone releasing hormone expression system in  
 which expression of gene encoding GHRH that induces production of  
 insulin-like froth factor-I in vivo, is not observed in absence of  
 ligand;  
 vector-mediated recombinant protein gene transfer and expression in  
 host cell for use in gene therapy  
 AU NORDSTROM J L; DRAGHIA-AKLI R  
 AN 2003-08687 BIOTECHDS  
 PI WO 2002097099 5 Dec 2002
- L61 ANSWER 10 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
 TI New transgenic animals (e.g. mice), useful as models for studying bone  
 density modulation, developing drugs for treating or preventing bone  
 diseases (e.g. osteoporosis), or diagnosing diseases characterized by  
 reduced bone density;

transgenic animal model construction and homologous recombination for use in gene therapy

AU BABIJ P; BEX F J; YAWORSKY P J; BODINE P V  
AN 2003-08268 BIOTECHDS  
PI WO 2002092764 21 Nov 2002

L61 ANSWER 11 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI New isolated nucleic acid representing a **synthetic BAX-gene**, useful as medicament for treating, preventing and/or alleviating yeast or fungal infections or proliferative disorders, or for preventing apoptosis in certain diseases;  
vector-mediated recombinant protein gene transfer and expression in *Candida sp.* cell for use in gene therapy, recombinant vaccine and nucleic acid vaccine preparation

AU CONTRERAS R H; EBERHARDT I; LUYTEN W H M L; REEKMAN R J  
AN 2003-02762 BIOTECHDS  
PI WO 2002064766 22 Aug 2002

L61 ANSWER 12 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Novel inducible expression system for regulating nucleic acid expression in gene therapy, provides undetectable biological effect or gene expression in absence of inducer, and **high expression** in presence of inducer;  
vector expression in host cell, and electroporation use in disease therapy and gene therapy

AU ABRUZZESE R V; MEHTA V; NORDSTROM J L  
AN 2003-00547 BIOTECHDS  
PI WO 2002024899 28 Mar 2002

L61 ANSWER 13 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Producing a recombinant adenoviral vector library useful for screening gene function, by transfecting a recombinant nucleic acid, and optionally adapter plasmid, into a cell with adenoviral E1-complementing sequences;  
virus vector expression in host cell and antisense sequence use in gene therapy

AU VOGELS R; BOUT A; VAN ES H H G; SHOUTEN G  
AN 2003-02583 BIOTECHDS  
PI US 6413776 2 Jul 2002

L61 ANSWER 14 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Synthesizing polynucleotide, by identifying initial and second polynucleotides, annealing initial sequence with second nucleotide, ligating above mixture, and repeating the steps for synthesizing the target;  
DNA primer and computer bioinformatic software for DNA synthesis

AU EVANS G A  
AN 2003-07226 BIOTECHDS  
PI WO 2002081490 17 Oct 2002

L61 ANSWER 15 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Synthesizing DNA sequences using a series of overlapping template oligonucleotides that have sequences corresponding to one strand of a DNA sequence and which are incapable of extension in the synthesis;  
vector-mediated gene transfer, expression in host cell and template DNA for DNA synthesis, genomics, nucleic acid vaccine and gene therapy

AU MCALISTER M; SAVVA R; BHATTACHARYA U  
AN 2002-17947 BIOTECHDS  
PI WO 2002050094 27 Jun 2002

L61 ANSWER 16 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Facilitating production of a protein for analyzing, designing and/or modifying an agent that can interact with a viral F protein, comprises expressing a nucleic acid optimized for expression of the protein, using a eukaryotic cell;

vector-mediated gene transfer and expression in host cell for  
recombinant vaccine and gene therapy

AU MASON A J; TUCKER S P; YOUNG P R  
AN 2003-01543 BIOTECHDS  
PI WO 2002042326 30 May 2002

L61 ANSWER 17 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Novel human cytomegalovirus Intron A fragment for use in expression  
constructs, lacks full-length Intron A sequence, and enhance expression  
levels when present in expression constructs;  
vector plasmid pCMVII-mediated recombinant protein gene transfer and  
expression in host cell for use in cancer diagnosis, prevention,  
therapy and gene therapy

AU THUDIUM K; SELBY M; ULMER J  
AN 2002-16517 BIOTECHDS  
PI WO 2002031137 18 Apr 2002

L61 ANSWER 18 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Screening for modulators of nuclear hormone receptor CAR, comprises  
administering a compound to a mouse expressing CAR and measuring  
induction of the CAR gene or measuring CAR gene expression in the  
presence and absence of the compound;  
drug screening, transgenic animal model, cell culture transfection and  
reporter gene expression

AU MOORE D D; WEI P; CHUA S S  
AN 2002-13289 BIOTECHDS  
PI WO 2002025270 28 Mar 2002

L61 ANSWER 19 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Recombinant polypeptide for immunizing a subject, comprises  
non-overlapping segments of amino acids identical to cytokine receptor  
sequences;

recombinant cytokine receptor and antibody for use in recombinant  
vaccine, therapy and gene therapy

AU PARHAM C L; GORMAN D M; KURATA H; ARAI N; SANA T R; MATTSON J D; MURPHY E  
E; SAVKOOR C; GREIN J; SMITH K M; MCCLANAHAN T K  
AN 2002-13069 BIOTECHDS  
PI WO 2002020569 14 Mar 2002

L61 ANSWER 20 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Novel nucleic acid comprising regions of rat progression elevated gene-3  
promoter for expressing foreign DNA in a host cell and treating  
melanoma, neuroblastoma, astrocytoma, cervical or breast cancer in  
humans;

retro virus vector-mediated gene transfer and expression in host for  
use in melanoma, neuroblastoma, cervical, lung, prostate, colon and  
glioblastoma cancer diagnostic, gene therapy and peptidomics

AU FISHER P B; SU Z  
AN 2002-11241 BIOTECHDS  
PI WO 2002008242 31 Jan 2002

L61 ANSWER 21 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
TI A synthetic DNA encoding an orange seapen Ptiloscarcus gurneyi-derived  
green fluorescent protein with codon preference of  
mammalian expression systems and biosensors

SO PCT Int. Appl., 77 pp.  
CODEN: PIXXD2

IN Chen, Yih-Tai; Cao, Longguang  
AN 2002:964489 HCAPLUS  
DN 138:34138

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002101020	A2	20021219	WO 2002-US18874	20020610
WO 2002101020	A3	20030807		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2002197673 A1 20021226 US 2001-977897 20011015

L61 ANSWER 22 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
 TI Modified vaccinia Ankara expressing modified HIV env, gag, and pol genes and uses in vaccination  
 SO PCT Int. Appl., 112 pp.  
 CODEN: PIXXD2

IN Moss, Bernard; Wyatt, Linda; Earl, Patricia  
 AN 2002:716417 HCAPLUS  
 DN 137:246528

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002072754	A2	20020919	WO 2002-US6713	20020301
WO 2002072754	C2	20030103		
WO 2002072754	A3	20030522		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L61 ANSWER 23 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
 TI Site saturation mutagenesis and polynucleotide reassembly and end selection in directed evolution  
 SO U.S., 114 pp., Cont.-in-part of U.S. Ser. No. 498,557.  
 CODEN: USXXAM

IN Short, Jay M.; Frey, Gerhard Johann  
 AN 2002:213739 HCAPLUS  
 DN 136:258264

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6358709	B1	20020319	US 2000-522289	20000309
US 5939250	A	19990817	US 1996-651568	19960522
US 5965408	A	19991012	US 1996-677112	19960709
US 5830696	A	19981103	US 1996-760489	19961205
US 6489145	B1	20021203	US 1997-962504	19971031
US 6335179	B1	20020101	US 1998-185373	19981103
US 6171820	B1	20010109	US 1999-246178	19990204
US 6238884	B1	20010529	US 1999-267118	19990309
US 6352842	B1	20020305	US 1999-276860	19990326
US 6537776	B1	20030325	US 1999-332835	19990614
US 6479258	B1	20021112	US 2000-495052	20000131
US 6361974	B1	20020326	US 2000-535754	20000327
AU 756201	B2	20030109	AU 2000-48933	20000731
AU 2000048933	A5	20001005		
US 6562594	B1	20030513	US 2001-756459	20010108
US 2002086279	A1	20020704	US 2001-875412	20010606
US 2002146762	A1	20021010	US 2001-885551	20010619
US 2003194763	A1	20031016	US 2002-99816	20020314
US 2003036116	A1	20030220	US 2002-108077	20020326



US 6635449 B2 20031021  
 US 2003219752 A1 20031127 US 2002-151469 20020517

- L61 ANSWER 24 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
 TI Genetic engineering of *Aspergillus awamori* for production of bovine chymosin (rennin)  
 SO Eur. Pat. Appl., 21 pp.  
 CODEN: EPXXDW
- IN Elena Cardoza, Rosa; Gutierrez Martin, Santiago; Moralejo Lorenzo, Francisco J.; Casqueiro Blanco, Francisco Javier; Martin Martin, Juan Francisco  
 AN 2002:611731 HCAPLUS  
 DN 137:168388
- |    | PATENT NO. | KIND | DATE     | APPLICATION NO. | DATE     |
|----|------------|------|----------|-----------------|----------|
| PI | EP 1231272 | A2   | 20020814 | EP 2002-380019  | 20020130 |
|    | EP 1231272 | A3   | 20021113 |                 |          |
- R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
- L61 ANSWER 25 OF 111 MEDLINE on STN DUPLICATE 10  
 TI Novel baculovirus DNA elements strongly stimulate activities of exogenous and endogenous **promoters**.  
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Feb 15) 277 (7) 5256-64.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 AU Lo Huei-Ru; Chou Cheng-Chung; Wu Tzong-Yuan; Yuen Joyce Pui-Yee; Chao Yu-Chan  
 AN 2002106153 MEDLINE
- L61 ANSWER 26 OF 111 MEDLINE on STN  
 TI Multiple pulmonary adenomas in the lung of transgenic mice overexpressing the RON receptor tyrosine kinase. Recepteur d'origine nantais.  
 SO CARCINOGENESIS, (2002 Nov) 23 (11) 1811-9.  
 Journal code: 8008055. ISSN: 0143-3334.  
 AU Chen Yi-Qing; Zhou Yong-Qing; Fu Lu-Hong; Wang Dong; Wang Ming-Hai  
 AN 2002684032 MEDLINE
- L61 ANSWER 27 OF 111 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 TI Long-term and tight control of gene expression in mouse skeletal muscle by a new hybrid human **transcription factor**  
 SO MOLECULAR THERAPY, (NOV 2002) Vol. 6, No. 5, pp. 653-663.  
 Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA.  
 ISSN: 1525-0016.  
 AU Roscilli G; Rinaudo C D; Cimino M; Sporeno E; Lamartina S; Ciliberto G; Toniatti C (Reprint)  
 AN 2002:934726 SCISEARCH
- L61 ANSWER 28 OF 111 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
 TI Tetracycline-inducible stable mammalian cell line **expression** systems for **high-level expression** of toxic rhodopsin **mutants**, non-glycosylated rhodopsin, and rhodopsin containing defined N-glycans.  
 SO FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A150. print.  
 Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology. New Orleans, Louisiana, USA. April 20-24, 2002.  
 CODEN: FAJOEC. ISSN: 0892-6638.  
 AU Reeves, Philip J. [Reprint author]; Callewaert, Nico; Contreras, Roland; Kim, Jong-Myoung [Reprint author]; Khorana, H. Gobind [Reprint author]  
 AN 2002:323154 BIOSIS
- L61 ANSWER 29 OF 111 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
 TI Diagnosing and treating glaucoma by analysis and control of the Frizzled Related Protein and Wnt pathway protein expression and activity.

PI WO 2001064949 A2 20010907 (200165)\* EN 74p C12Q001-68  
 RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR  
 W: AU BR CA CN JP KR MX PL ZA  
 AU 2001041768 A 20010912 (200204) C12Q001-68  
 US 2002049177 A1 20020425 (200233) A61K048-00  
 EP 1259648 A2 20021127 (200302) EN C12Q001-68  
 R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR  
 KR 2003005212 A 20030117 (200334) C12Q001-68  
 CN 1426482 A 20030625 (200362) C12Q001-68  
 MX 2002008487 A1 20030101 (200373) A01K067-027  
 IN CLARK, A F; FINGERT, J; MCNATT, L; STONE, E; WANG, W; STONE, E M

L61 ANSWER 30 OF 111 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
 TI New isolated **mutated** human p53 polypeptides for inducing  
 toxicity in a cell, treating cancer and identifying compounds that mimic  
 toxic or supertransactivating **mutations**.  
 PI WO 2001009325 A2 20010208 (200113)\* EN 144p C12N015-12  
 RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ  
 NL OA PT SD SE SL SZ TZ UG ZW  
 W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM  
 DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC  
 LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE  
 SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW  
 AU 2000062395 A 20010219 (200129) C12N015-12  
 EP 1204745 A2 20020515 (200239) EN C12N015-12  
 R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT  
 RO SE SI  
 JP 2003506041 W 20030218 (200315) 151p C12N015-09  
 IN INGA, A; RESNICK, M A

L61 ANSWER 31 OF 111 MEDLINE on STN DUPLICATE 11  
 TI Deletion of RAR carboxyl terminus reveals **promoter**- and  
 receptor-specific AP-1 effects.  
 SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2001 Dec 21) 289 (5)  
 950-6.  
 Journal code: 0372516. ISSN: 0006-291X.  
 AU Aneskievich B J  
 AN 2001700851 MEDLINE

L61 ANSWER 32 OF 111 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN DUPLICATE  
 12  
 TI A rapid and simple method for construction and expression of a  
**synthetic** human growth hormone **gene** in Escherichia coli  
 SO JOURNAL OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, (30 NOV 2001) Vol. 34, No.  
 6, pp. 502-508.  
 Publisher: SPRINGER-VERLAG SINGAPORE PTE LTD, #04-01 CENCON I, 1 TANNERY  
 RD, SINGAPORE 347719, SINGAPORE.  
 ISSN: 1225-8687.  
 AU Roytrakul S; Euxwilaichitr L (Reprint); Suprasongsin C; Panyim S  
 AN 2001:961866 SCISEARCH

L61 ANSWER 33 OF 111 MEDLINE on STN DUPLICATE 13  
 TI Overexpression of artificial **synthetic gene** of  
 Aspergillus niger NRRL3135 phytase in Pichia pastoris.  
 SO SHENG WU KUNG CH ENG HSUEH PAO, (2001 May) 17 (3) 254-8.  
 Journal code: 9426463. ISSN: 1000-3061.  
 AU Bei J L; Chen Z; Yang L; Liao L; Wang X Z; Jiang Z Y  
 AN 2001474246 MEDLINE

L61 ANSWER 34 OF 111 MEDLINE on STN DUPLICATE 14  
 TI Reduction of wobble-position GC bases in Corynebacteria genes and  
 enhancement of PCR and heterologous expression.  
 SO JOURNAL OF MOLECULAR MICROBIOLOGY AND BIOTECHNOLOGY, (2001 Jan) 3 (1)  
 123-6.

Journal code: 100892561. ISSN: 1464-1801.

AU Sanli G; Blaber S I; Blaber M  
AN 2001459358 MEDLINE

L61 ANSWER 35 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Modified **synthetic DNA sequences** comprise  
modification of the truncated cry9Aa **gene** of *Bacillus*  
*thuringiensis* for improved insect control in plants;  
transgenic plant construction with improved disease-resistance  
AU Kuvshinov V; Kanerva A; Koivu K; Pehu E  
AN 2000-06780 BIOTECHDS  
PI WO 2000011025 2 Mar 2000

L61 ANSWER 36 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
TI CDX2 is downstream mediator of APC tumor suppressor activity  
SO PCT Int. Appl., 27 pp.  
CODEN: PIXXD2  
IN Dacosta, Luis; Vogelstein, Bert; Kinzler, Kenneth W.; He, Tong-chuan  
AN 2000:824448 HCAPLUS  
DN 134:1380

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000070089	A1	20001123	WO 2000-US12893	20000512
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
US 6511799	B1	20030128	US 1999-311551	19990514

L61 ANSWER 37 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
TI Generation of genetic vaccines and immunomodulatory polynucleotides by non-stochastic directed evolution techniques  
SO PCT Int. Appl., 718 pp.  
CODEN: PIXXD2  
IN Short, Jay M.  
AN 2000:553678 HCAPLUS  
DN 133:160529

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000046344	A2	20000810	WO 2000-US3086	20000204
WO 2000046344	A3	20001228		
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
US 6171820	B1	20010109	US 1999-246178	19990204
EP 1073710	A2	20010207	EP 2000-913378	20000204
EP 1073710	A3	20010307		
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO			
JP 2003524392	T2	20030819	JP 2000-597406	20000204
AU 756201	B2	20030109	AU 2000-48933	20000731
AU 2000048933	A5	20001005		
US 2002086279	A1	20020704	US 2001-875412	20010606

- L61 ANSWER 38 OF 111 MEDLINE on STN DUPLICATE 16  
 TI Runt domain factor (Runx)-dependent effects on CCAAT/ enhancer-binding protein delta expression and activity in osteoblasts.  
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jul 14) 275 (28) 21746-53.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 AU McCarthy T L; Ji C; Chen Y; Kim K K; Imagawa M; Ito Y; Centrella M  
 AN 2000396738 MEDLINE
- L61 ANSWER 39 OF 111 MEDLINE on STN DUPLICATE 17  
 TI The Pezcoller lecture: cancer cell cycles revisited.  
 SO CANCER RESEARCH, (2000 Jul 15) 60 (14) 3689-95. Ref: 118  
 Journal code: 2984705R. ISSN: 0008-5472.  
 AU Sherr C J  
 AN 2000402340 MEDLINE
- L61 ANSWER 40 OF 111 LIFESCI COPYRIGHT 2003 CSA on STN  
 TI CamKII alpha -cre Transgene Expression and Recombination Patterns in the Mouse Brain  
 SO Genesis, (20000200) vol. 26, no. 2, pp. 133-135. Special Issue: Issue Specific Expression of Cre Recombinase in Mice..  
 ISSN: 1526-954X.  
 AU Dragatsis, I.; Zeitlin, S.  
 AN 2000:66593 LIFESCI
- L61 ANSWER 41 OF 111 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
 TI Beyond the full-length gene barrier: The large-scale production of synthetic genes.  
 SO International Genome Sequencing and Analysis Conference, (2000) Vol. 12, pp. 72. print.  
 Meeting Info.: 12th International Genome Sequencing and Analysis Conference. Miami Beach, Florida, USA. September 12-15, 2000.  
 AU Kittle, Joseph D., Jr. [Reprint author]  
 AN 2001:514716 BIOSIS
- L61 ANSWER 42 OF 111 MEDLINE on STN DUPLICATE 18  
 TI Codon optimization of xylanase gene xynB from the thermophilic bacterium Dictyoglomus thermophilum for expression in the filamentous fungus Trichoderma reesei.  
 SO FEMS MICROBIOLOGY LETTERS, (2000 Sep 1) 190 (1) 13-9.  
 Journal code: 7705721. ISSN: 0378-1097.  
 AU Te'o V S; Cziferszky A E; Bergquist P L; Nevalainen K M  
 AN 2001096599 MEDLINE
- L61 ANSWER 43 OF 111 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
 TI New flowering locus T polypeptide that regulates flowering time, particularly used to accelerate flowering.  
 PI WO 9953070 A1 19991021 (199952)\* EN 63p C12N015-29  
 RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL  
 OA PT SD SE SL SZ UG ZW  
 W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB  
 GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU  
 LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR  
 TT UA UG UZ VN YU ZA ZW  
 AU 9935601 A 19991101 (200013)  
 EP 1073743 A1 20010207 (200109) EN C12N015-29  
 R: AT BE CH CY DE DK ES FI FR GB GR IE LI LU MC NL PT SE  
 US 6225530 B1 20010501 (200126) C12N015-29  
 CN 1302328 A 20010704 (200158) C12N015-29  
 BR 9910123 A 20011002 (200167) C12N015-29  
 KR 2001042756 A 20010525 (200168) C07K014-415  
 US 2001049831 A1 20011206 (200203) C12N005-02  
 US 2002029395 A1 20020307 (200221) C12N015-82

JP 2002511270 W 20020416 (200242) 62p C12N015-09  
AU 757842 B 20030306 (200324) C12N015-29  
NZ 507854 A 20030530 (200341) C12N015-29  
IN WEIGEL, D; KARDAILSKY, I

L61 ANSWER 44 OF 111 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
TI New human lectomedin receptor polypeptide, used to identify specific binding partners for treating e.g. vascular disease.  
PI WO 9945111 A1 19990910 (199948)\* EN 166p C12N015-12  
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL  
OA PT SD SE SL SZ UG ZW  
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD  
GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV  
MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT  
UA UG UZ VN YU ZW  
AU 9929807 A 19990920 (200007) C12N015-12  
EP 1060248 A1 20001220 (200105) EN C12N015-12  
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE  
JP 2002505104 W 20020219 (200216) 174p C12N015-09  
US 6479256 B1 20021112 (200278) C12P021-06  
IN HAYFLICK, J S

L61 ANSWER 45 OF 111 MEDLINE on STN  
TI Development of viral vectors for gene therapy of beta-chain hemoglobinopathies: optimization of a gamma-globin gene expression cassette.  
SO BLOOD, (1999 Apr 1) 93 (7) 2208-16.  
Journal code: 7603509. ISSN: 0006-4971.  
AU Li Q; Emery D W; Fernandez M; Han H; Stamatoyannopoulos G  
AN 1999192436 MEDLINE

L61 ANSWER 46 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
TI Study on the expression of recombinant human thrombopoietin in Escherichia coli  
SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1999), 15(1), 19-23  
CODEN: ZSHXFF2; ISSN: 1007-7626  
AU Zhao, Dong; Lu, Baisong; Chen, Lin; Liu, Xiaolan; Huang, Peitang  
AN 1999:202170 HCAPLUS  
DN 131:1231

L61 ANSWER 47 OF 111 MEDLINE on STN DUPLICATE 19  
TI Gene synthesis by a LCR-based approach: high-level production of leptin-L54 using **synthetic gene** in Escherichia coli.  
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Jul 9) 248 (1) 200-3.  
Journal code: 0372516. ISSN: 0006-291X.  
AU Au L C; Yang F Y; Yang W J; Lo S H; Kao C F  
AN 1998340875 MEDLINE

L61 ANSWER 48 OF 111 LIFESCI COPYRIGHT 2003 CSA on STN  
TI Transcriptional Regulation of the Human Proenkephalin Gene by Conformational Switching: Implications for Decoy Design  
SO Antisense Nucleic Acid Drug Dev., (19980400) vol. 8, no. 2, pp. 159-165.  
ISSN: 1087-2906.  
AU Spiro, C.; McMurray, C.T.\*  
AN 1998:97956 LIFESCI

L61 ANSWER 49 OF 111 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN DUPLICATE 20  
TI Instability of Aspergillus niger glucoamylase cDNA in a high copy number vector cloned into Escherichia coli  
SO ASIA-PACIFIC JOURNAL OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY, (JUN 1998) Vol. 6, No. 1, pp. 39-46.  
Publisher: UNIV MALAYA, INST POSTGRADUATE STUDIES & RESEARCH, KUALA LUMPUR

50603, MALAYSIA.

ISSN: 0128-7451.

AU Abdulrashid N (Reprint); Hartley B S

AN 1998:677932 SCISEARCH

L61 ANSWER 50 OF 111 MEDLINE on STN

TI A point **mutation** within CD45 exon A is the cause of variant CD45RA splicing in humans.

SO EUROPEAN JOURNAL OF IMMUNOLOGY, (1998 Jan) 28 (1) 22-9.

Journal code: 1273201. ISSN: 0014-2980.

AU Zilch C F; Walker A M; Timon M; Goff L K; Wallace D L; Beverley P C

AN 1998143724 MEDLINE

L61 ANSWER 51 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN

TI **Synthetic HIV genes** for use in vector vaccines

SO PCT Int. Appl., 82 pp.

CODEN: PIXXD2

IN Shiver, John W.; Davies, Mary-Ellen; Freed, Daniel C.; Liu, Margaret A.; Perry, Helen C.

AN 1997:579828 HCAPLUS

DN 127:247095

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI WO 9731115	A2	19970828	WO 1997-US2294	19970218
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WO 9731115	A3	19971009		
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W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9721246	A1	19970910	AU 1997-21246	19970218
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AU 729231	B2	20010125		
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EP 904380	A2	19990331	EP 1997-906594	19970218
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, SI, LT, LV, FI, RO

BR 9707672	A	19990413	BR 1997-7672	19970218
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CN 1216064	A	19990505	CN 1997-193817	19970218
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NZ 331161	A	20000327	NZ 1997-331161	19970218
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JP 2000505299	T2	20000509	JP 1997-530231	19970218
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ZA 9701449	A	19970822	ZA 1997-1449	19970220
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NO 9803876	A	19981021	NO 1998-3876	19980821
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US 6534312	B1	20030318	US 1999-340798	19990628
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US 2003087225	A1	20030508	US 2002-41414	20020520
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US 2003229214	A1	20031211	US 2003-369121	20030217
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L61 ANSWER 52 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN

TI Recombinant nucleic acids containing a negative transdominant **mutant** of gene rev for inhibiting HIV gene expression

SO U.S., 35 pp.

CODEN: USXXAM

IN Nabel, Gary J.; Yang, Zhi-yong; Liu, Jinsong; Woffendin, Clive

AN 1997:492879 HCAPLUS

DN 127:186605

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI US 5650306	A	19970722	US 1993-73836	19930607
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L61 ANSWER 53 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN

TI Inhibitor-resistant urokinase and transgenic animals secreting the enzyme into the milk

SO U.S., 16 pp., Cont. of U. S. Ser. No. 631,673, abandoned.

CODEN: USXXAM

IN Wei, Cha Mer  
AN 1997:492809 HCAPLUS  
DN 127:146512

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5648253	A	19970715	US 1992-942157	19920908

L61 ANSWER 54 OF 111 MEDLINE on STN DUPLICATE 21  
TI Specific sequence modifications of a cry3B endotoxin gene result in high levels of **expression** and insect resistance.  
SO PLANT MOLECULAR BIOLOGY, (1997 Jun) 34 (3) 485-96.  
Journal code: 9106343. ISSN: 0167-4412.  
AU Iannacone R; Grieco P D; Cellini F  
AN 97369371 MEDLINE

L61 ANSWER 55 OF 111 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
TI Proteolytic activity in vivo and encapsidation of recombinant human immunodeficiency virus type 1 proteinase expressed in baculovirus-infected cells  
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Publisher: SOC GENERAL MICROBIOLOGY, HARVEST HOUSE 62 LONDON ROAD, READING, BERKS, ENGLAND RG1 5AS.  
ISSN: 0022-1317.  
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AN 97:91145 SCISEARCH

L61 ANSWER 56 OF 111 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V. on STN  
AN 1997119200 ESBIOWASE  
TI Expression of de novo high-lysine  $\alpha$ -helical coiled-coil proteins may significantly increase the accumulated levels of lysine in mature seeds of transgenic tobacco plants  
AU Keeler S.J.; Maloney C.L.; Webber P.Y.; Patterson C.; Hirata L.T.; Falco S.C.; Rice J.A.  
CS S.J. Keeler, Department of Plant/Soil Sciences, 149 Townsend Hall, University of Delaware, Newark, DE 19717, United States.  
SO Plant Molecular Biology, (1997), 34/1 (15-29), 55 reference(s)  
CODEN: PMBIDB ISSN: 0167-4412  
DT Journal; Article  
CY Netherlands  
LA English  
SL English

L61 ANSWER 57 OF 111 MEDLINE on STN DUPLICATE 22  
TI Structure and function in rhodopsin: **high level expression of a synthetic bovine opsin gene** and its **mutants** in stable mammalian cell lines.  
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Journal code: 7505876. ISSN: 0027-8424.  
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AN 97030221 MEDLINE

L61 ANSWER 58 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Cloning and characterization of cDNA encoding farnesyl-diphosphate-synthase from rubber tree; potential crop improvement  
SO Plant Mol.Biol.; (1996) 30, 5, 935-46  
CODEN: PMBIDB ISSN: 0167-4412  
AU Adiwilaga K; \*Kush A  
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L61 ANSWER 59 OF 111 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN DUPLICATE 23

TI Optimization of the bovine growth hormone gene expression in E-coli  
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L61 ANSWER 60 OF 111 MEDLINE on STN DUPLICATE 24  
 TI A novel Escherichia coli vector for oxygen-inducible **high level**  
**expression** of foreign genes.  
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 Journal code: 7706761. ISSN: 0378-1119.  
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L61 ANSWER 61 OF 111 MEDLINE on STN DUPLICATE 25  
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 49-55.  
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L61 ANSWER 77 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
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CODEN: PIXXD2  
IN Shapiro, David J.; Nielsen, David A.; Kemper, Byron W.; Szczesna-Skorupa, Elzbieta; Xing, Hong  
AN 1992:209130 HCAPLUS  
DN 116:209130

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9202529	A1	19920220	WO 1991-US5404	19910730
W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, PL, RO, SD, SE, SU				
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG				
AU 9184160	A1	19920302	AU 1991-84160	19910730

L61 ANSWER 78 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
TI Human hemoglobin expression in Escherichia coli: importance of optimal codon usage;  
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SO Biochemistry; (1992) 31, 36, 8619-28  
CODEN: BICHAW  
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L61 ANSWER 79 OF 111 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
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L61 ANSWER 82 OF 111 MEDLINE on STN DUPLICATE 30  
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 SO GENE, (1992 Dec 15) 122 (2) 263-9.  
 Journal code: 7706761. ISSN: 0378-1119.  
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L61 ANSWER 83 OF 111 MEDLINE on STN  
 TI **Synthetic cryIIIA gene** from Bacillus thuringiensis improved for **high expression** in plants.  
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 Journal code: 9209120. ISSN: 0962-8819.  
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L61 ANSWER 84 OF 111 MEDLINE on STN DUPLICATE 31  
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 Journal code: 7706761. ISSN: 0378-1119.  
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L61 ANSWER 85 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
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 CODEN: PIXXD2  
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 AN 1992:1768 HCAPLUS  
 DN 116:1768

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9114785	A1	19911003	WO 1991-US2186	19910328
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
US 5143829	A	19920901	US 1990-501206	19900329
AU 9176774	A1	19911021	AU 1991-76774	19910328
AU 660394	B2	19950622		
EP 522080	A1	19930113	EP 1991-908053	19910328
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
JP 05508075	T2	19931118	JP 1991-507624	19910328
JP 3176916	B2	20010618		
JP 2001169793	A2	20010626	JP 2000-353649	19910328
CA 2078875	C	20020226	CA 1991-2078875	19910328

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TI A new family of sugar-inducible expression vectors for *Escherichia coli*.  
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USE OF A DEGENERATIVE MIXTURE OF SYNTHETIC RIBOSOME BINDING-SITES AND  
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CODEN: GENED6; ISSN: 0378-1119  
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DN 116:1654
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Journal code: 8109087. ISSN: 0270-7306.  
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Journal code: 7505876. ISSN: 0027-8424.  
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L61 ANSWER 94 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
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 tendamistat gene cloning in *Escherichia coli* and *Streptomyces*  
*lividans*; gene amplification; DNA sequence  
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 CODEN: JOBAAY  
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L61 ANSWER 95 OF 111 MEDLINE on STN DUPLICATE 36  
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 Journal code: 8109087. ISSN: 0270-7306.  
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L61 ANSWER 96 OF 111 MEDLINE on STN DUPLICATE 37  
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L61 ANSWER 97 OF 111 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
 DUPLICATE 38  
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 AN 1990:131339 BIOSIS

L61 ANSWER 98 OF 111 MEDLINE on STN  
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 Journal code: 7706761. ISSN: 0378-1119.  
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L61 ANSWER 99 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
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 plasmids containing these genes, and **high-level**  
**expression** of the genes in bacteria  
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 CODEN: EPXXDW  
 IN Zurawski, Gerard; Zurawski, Sandra Marvo  
 AN 1988:449634 HCAPLUS  
 DN 109:49634

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 259160	A2	19880309	EP 1987-307781	19870903
EP 259160	A3	19881130		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
US 5017692	A	19910521	US 1986-903497	19860904
EP 481536	A1	19920422	EP 1991-122275	19870903
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
JP 63071185	A2	19880331	JP 1987-221809	19870904

L61 ANSWER 100 OF 111 MEDLINE on STN DUPLICATE 39

TI Protein engineering of antibody binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in *Escherichia coli*.

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L61 ANSWER 101 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN

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protease-inhibitor SLPI expression in *Escherichia coli* (conference abstract)

SO J.Cell.Biochem.; (1988) Suppl.12D, 70

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L61 ANSWER 102 OF 111 MEDLINE on STN DUPLICATE 40

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Journal code: 2985121R. ISSN: 0021-9258.

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L61 ANSWER 103 OF 111 MEDLINE on STN DUPLICATE 41

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SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1987 Mar 15) 262 (8) 3609-14.  
Journal code: 2985121R. ISSN: 0021-9258.

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L61 ANSWER 104 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN

TI Chemical synthesis and in vivo hyperexpression of a modular gene coding for *Escherichia coli* translational initiation factor IF1;  
vector construction and cloning (conference abstract)

SO Protein Eng.; (1987) 1, 3, 253  
CODEN: PRENE9

AU Calogero R A; Pon C L; Gualerzi C O

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L61 ANSWER 105 OF 111 MEDLINE on STN DUPLICATE 42

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Journal code: 0125036. ISSN: 0026-8925.

AU Calogero R A; Pon C L; Gualerzi C O

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L61 ANSWER 106 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN

TI Expression of murine epidermal growth factor in *Escherichia coli*;  
**synthetic gene** expression and vector construction  
(conference abstract)

SO Biol.Chem.Hoppe Seyler; (1986) 367, Suppl., 162  
CODEN: BCHSEI

AU Allen G; Henwood C A; Winther M D

AN 1986-11861 BIOTECHDS

L61 ANSWER 107 OF 111 MEDLINE on STN DUPLICATE 43

TI **High-level expression** of a gene encoding the human complement factor C5a in *Escherichia coli*.

SO GENE, (1986) 43 (1-2) 131-8.  
Journal code: 7706761. ISSN: 0378-1119.

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L61 ANSWER 108 OF 111 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN  
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CODEN: FEPR47  
AU Shatzman A R; Rosenberg M  
AN 1985-05769 BIOTECHDS

L61 ANSWER 109 OF 111 MEDLINE on STN DUPLICATE 44  
TI Expression of a **synthetic** human growth hormone gene in  
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SO GENE, (1985) 39 (1) 117-20.  
Journal code: 7706761. ISSN: 0378-1119.  
AU Tokunaga T; Iwai S; Gomi H; Kodama K; Ohtsuka E; Ikehara M; Chisaka O;  
Matsubara K  
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L61 ANSWER 110 OF 111 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 45  
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America, (1984) Vol. 81, No. 16, pp. 5056-5060.  
CODEN: PNASA6. ISSN: 0027-8424.  
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L61 ANSWER 111 OF 111 HCAPLUS COPYRIGHT 2003 ACS on STN  
TI The manufacture and expression of structural genes  
SO PCT Int. Appl., 46 pp.  
CODEN: PIXXD2  
IN Stabinsky, Yitzhak  
AN 1984:133585 HCAPLUS  
DN 100:133585

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 8304029	A1	19831124	WO 1983-US563	19830415
W: JP				
RW: AT, BE, CH, DE, FR, GB, LU, NL, SE				
US 4652639	A	19870324	US 1982-375493	19820506
EP 108787	A1	19840523	EP 1983-901773	19830415
EP 108787	B1	19900411		
R: AT, BE, CH, DE, FR, GB, LI, LU, NL, SE				
JP 59501096	T2	19840628	JP 1983-501807	19830415
JP 07089934	B4	19951004		
AT 51873	E	19900415	AT 1983-901773	19830415
IL 68491	A1	19900726	IL 1983-68491	19830426
CA 1266628	A1	19900313	CA 1983-427371	19830504

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COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

163.37

163.58

STN INTERNATIONAL LOGOFF AT 16:04:46 ON 16 DEC 2003

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	32075	(humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1)	USPAT; US-PGPUB	2003/12/16 11:41
2	L2	8	1 same (regulat\$8 and codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:45
3	L3	244	(transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon adj preference	USPAT; US-PGPUB	2003/12/16 14:50
4	L4	175	3 and 1	USPAT; US-PGPUB	2003/12/16 11:55
5	L5	353	1 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:40
6	L6	3893	1 same (regulat\$8 or codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:08
7	L7	6442	1 same muta\$10	USPAT; US-PGPUB	2003/12/16 14:08
8	L8	1942	6 and 7	USPAT; US-PGPUB	2003/12/16 14:08
9	L9	52	8 and 5	USPAT; US-PGPUB	2003/12/16 14:09
10	L10	0	3 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:50
11	L11	186	3 and (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:43
12	L12	0	3 and 5	USPAT; US-PGPUB	2003/12/16 14:43
13	L13	7	(luciferase\$1 or gfp) same (codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:46
14	L14	12712	(transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon	USPAT; US-PGPUB	2003/12/16 14:50
15	L15	384	14 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:51
16	L16	7469	14 and 1	USPAT; US-PGPUB	2003/12/16 14:51
17	L17	192	15 and 1	USPAT; US-PGPUB	2003/12/16 14:52
18	L18	78	15 and 7	USPAT; US-PGPUB	2003/12/16 14:52



	L #	Hits	Search Text	DBs	Time Stamp
1	L1	32075	(humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1)	USPAT; US-PGPUB	2003/12/16 11:41
2	(L2)	8	1 same (regulat\$8 and codon adj preference)	USPAT; US-PGPUB	2003/12/16 11:42

PGPUB-DOCUMENT-NUMBER: 20030228329

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030228329 A1

TITLE: Adenovirus carrying gag gene HIV vaccine

PUBLICATION-DATE: December 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chen, Ling	Blue Bell	PA	US	
Shiver, John W.	Doylestown	PA	US	
Bett, Andrew J.	Lansdale	PA	US	
Casimiro, Danilo R.	Harleysville	PA	US	
Caulfield, Michael J.	Fort Washington	PA	US	
Chastain, Michael A.	Glenside	PA	US	
Emini, Emilio A.	Strafford	PA	US	

APPL-NO: 10/ 461030

DATE FILED: June 13, 2003

RELATED-US-APPL-DATA:

child 10461030 A1 20030613

parent continuation-of 09818443 20010327 US PENDING

child 09818443 20010327 US

parent continuation-in-part-of PCT/US00/18332 20000703 US PENDING

non-provisional-of-provisional 60148981 19990813 US

non-provisional-of-provisional 60142631 19990706 US

US-CL-CURRENT: 424/199.1, 435/235.1 , 435/456

ABSTRACT:

An adenoviral vector is described which carries a codon-optimized gag gene, along with a heterologous promoter and transcription terminator. This viral vaccine can effectively prevent HIV infection when administered to humans either alone or as part of a prime and boost regime also with a vaccine plasmid.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of PCT International

Application No. PCT/US00/18332, filed Jul. 3, 2000, which designates the U.S., which claims the benefit, under 35 U.S.C. .sctn.119(e), of U.S. Provisional Application Serial No. 60/148,981, filed Aug. 13, 1999 and U.S. Provisional Application Serial No. 60/142,631, filed Jul. 6, 1999.

----- KWIC -----

Brief Description of Drawings Paragraph - DRTX (37):

[0054] The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector which specifically includes a gag gene which is codon optimized for expression in a human cellular environment.

Detail Description Paragraph - DETX (26):

[0096] Construction of FG adenovirus FL gag. The full-length (FL) humanized gag gene was ligated into an adenovirus-5 shuttle vector, pHCMVIBGHpA1, containing Ad5 sequences from bp 1 to bp 341 and bp 3534 to bp 5798 with a expression cassette containing human CMV promoter plus intron A and bovine growth hormone polyadenylation signal. The orientation was confirmed by restriction enzyme digestion analysis and DNA sequencing. Homologous recombination in E. coli was employed using the shuttle plasmid, pA1-CMV1-FLHIVgag, and adenoviral backbone plasmid, pAdE1-E3-, to generate a plasmid form of the recombinant adenovirus containing the expression regulatory elements and FL gag gene, pAd.CMV1-FHIVgag. Appropriate plasmid recombinants were confirmed by restriction enzyme digestion.

PGPUB-DOCUMENT-NUMBER: 20030138782

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138782 A1

TITLE: Computer-directed assembly of a polynucleotide encoding  
a target polypeptide

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Evans, Glen A.	San Marcos	CA	US	

APPL-NO: 10/ 052582

DATE FILED: January 18, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60262693 20010119 US

US-CL-CURRENT: 435/6, 435/91.2 , 702/20

ABSTRACT:

The present invention outlines a novel approach to utilizing the results of genomic sequence information by computer-directed polynucleotide assembly based upon information available in databases such as the human genome database. Specifically, the present invention may be used to select, synthesize and assemble a novel, synthetic target polynucleotide sequence encoding a target polypeptide. The target polynucleotide may encode a target polypeptide that exhibits enhanced or altered biological activity as compared to a model polypeptide encoded by a natural (wild-type) or model polynucleotide sequence.

[0001] This application is based on, and claims the benefit of, U.S. Provisional Application No. 60/262,693, filed Jan. 19, 2001, and entitled COMPUTER-DIRECTED ASSEMBLY OF A POLYNUCLEOTIDE ENCODING A TARGET POLYPEPTIDE, and which is incorporated herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX (21):

[0056] The present invention provides also provides methods that can be used to synthesize, de novo, polynucleotides that encode sets of genes, either naturally occurring genes expressed from natural or artificial promoter constructs or artificial genes derived from synthetic DNA sequences, which

encode elements of biological systems that perform a specified function or attribution of an artificial organism as well as entire genomes. In producing such systems and genomes, the present invention provides the synthesis of a replication-competent, double-stranded polynucleotide, wherein the polynucleotide has an origin of replication, a first coding region and a first **regulatory** element directing the expression of the first coding region. By replication competent, it is meant that the polynucleotide is capable of directing its own replication. Thus, it is envisioned that the polynucleotide will possess all the cis-acting signals required to facilitate its own synthesis. In this respect, the polynucleotide will be similar to a plasmid or a virus, such that once placed within a cell, it is capable of replication by a combination of the polynucleotide's and cellular functions.

Detail Description Paragraph - DETX (182):

[0216] For the purposes of assembling a **synthetic nucleic acid sequence** encoding a target polypeptide, a model polypeptide sequence or nucleic acid sequence is obtained and analyzed using a suitable DNA analysis package, such as, for example, MacVector or DNA Star. If the target protein will be expressed in a bacterial system, for example, the model sequence can be converted to a sequence encoding a polypeptide utilizing E. coli preferred codons (i.e., Type I, Type II or Type II **codon preference**). The present invention provides the conversion programs Codon I, Codon II or Codon III. A nucleic acid sequence of the invention can be designed to accommodate any **codon preference** of any prokaryotic or eucaryotic organism.

PGPUB-DOCUMENT-NUMBER: 20030087238

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087238 A1

TITLE: Method for assembly of a polynucleotide encoding a target polypeptide

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Evans, Glen A.	San Marcos	CA	US	

APPL-NO: 09/ 922221

DATE FILED: August 2, 2001

RELATED-US-APPL-DATA:

child 09922221 A1 20010802

parent continuation-in-part-of 09554929 20000512 US PENDING

child 09554929 20000512 US

parent a-371-of-international PCT/US98/19312 19980916 WO UNKNOWN

non-provisional-of-provisional 60059017 19970916 US

US-CL-CURRENT: 435/6, 435/91.2 , 536/25.3

ABSTRACT:

The present invention provides a novel approach to utilizing the results of genomic sequence information by computer-directed polynucleotide assembly based upon information available in databases such as the human genome database. Specifically, the present invention can be used to select, synthesize and assemble a novel, synthetic target polynucleotide sequence encoding a target polypeptide. The target polynucleotide can encode a target polypeptide that exhibits enhanced or altered biological activity as compared to a model polypeptide encoded by a natural (wild-type) or model polynucleotide sequence.

----- KWIC -----

Detail Description Paragraph - DETX (55):

[0080] The present invention provides also provides methods that can be used to synthesize, de novo, polynucleotides that encode sets of genes, either

naturally occurring genes expressed from natural or artificial promoter constructs or artificial **genes derived from synthetic DNA sequences**, which encode elements of biological systems that perform a specified function or attribution of an artificial organism as well as entire genomes. In producing such systems and genomes, the present invention provides the synthesis of a replication-competent, double-stranded polynucleotide, wherein the polynucleotide has an origin of replication, a first coding region and a first **regulatory** region directing the expression of the first coding region.

Detail Description Paragraph - DETX (166):

[0190] For the purposes of assembling a **synthetic nucleic acid sequence** encoding a target polypeptide, a model polypeptide sequence or nucleic acid sequence is obtained and analyzed using a suitable DNA analysis package, such as, for example, MacVector or DNA Star. If the target protein will be expressed in a bacterial system, for example, the model sequence can be converted to a sequence encoding a polypeptide utilizing E. coli preferred codons (i.e., Type I, Type II or Type II **codon preference**). The present invention provides the conversion programs Codon I, Codon II or Codon III. However, a nucleic acid sequence of the invention can be designed to accommodate any **codon preference** of any prokaryotic or eukaryotic organism.

US-PAT-NO: 6645761

DOCUMENT-IDENTIFIER: US 6645761 B1

TITLE: Humanized polynucleotide sequence encoding Renilla  
mulleri green fluorescent protein

DATE-ISSUED: November 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sorge; Joseph A.	Wilson	WY	N/A	N/A
Vaillancourt; Peter Edward	Del Mar	CA	N/A	N/A

APPL-NO: 09/ 839650

DATE FILED: April 19, 2001

PARENT-CASE:

This application is a continuation-in-part of U.S. patent application Ser. No. 09/748,786, filed Dec. 22, 2000, now abandoned, incorporated herein by reference in its entirety.

US-CL-CURRENT: 435/325, 435/243 , 435/320.1 , 435/410 , 536/23.5

ABSTRACT:

The present invention provides a polynucleotide encoding a green fluorescent protein from Renilla mulleri comprising a humanized sequence which permits enhanced expression of the encoded polypeptide in mammalian cells.

3 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

----- KWIC -----

Brief Summary Text - BSTX (26):

The invention further provides a method of monitoring the activity of a transcriptional regulatory sequence, the method comprising the steps of: operably linking a nucleic acid sequence comprising the transcriptional regulatory sequence to a humanized nucleic acid sequence encoding R. mulleri GFP to form a reporter construct; introducing the reporter construct to a cell; and detecting R. mulleri GFP fluorescence in the cell, wherein the fluorescence reflects the activity of the transcriptional regulatory sequence.



Brief Summary Text - BSTX (27):

The invention still further provides a method of detecting a modulator of a transcriptional **regulatory** sequence, the method comprising the steps of: operably linking a nucleic acid **sequence comprising the transcriptional regulatory sequence to a humanized nucleic acid sequence** encoding R. mulleri GFP to form a reporter construct, wherein the transcriptional **regulatory** sequence is responsive to the presence of the modulator; introducing the reporter construct to a cell; and detecting R. mulleri GFP fluorescence in the cell, wherein the fluorescence indicates the presence of the modulator.

Brief Summary Text - BSTX (28):

The invention still further provides a method of screening for an inhibitor of a transcriptional **regulatory** sequence, the method comprising the steps of: operably linking a nucleic acid **sequence comprising the transcriptional regulatory sequence to a humanized nucleic acid sequence** encoding R. mulleri GFP to form a reporter construct; introducing the reporter construct to a cell; contacting the cell with a candidate inhibitor of the transcriptional **regulatory** sequence; and detecting R. mulleri GFP fluorescence in the cell, wherein a decrease in the fluorescence relative to that detected in the absence of the candidate inhibitor indicates that the candidate inhibitor inhibits the activity of the transcriptional **regulatory** sequence.

Brief Summary Text - BSTX (35):

As used herein, the term "**humanized codon**" means a codon, within a **polynucleotide sequence** encoding a non-human polypeptide, that has been changed to a codon that is more preferred for expression in human cells relative to that codon encoded by the non-human organism from which the non-human polypeptide is derived. Species-specific codon preferences stem in part from differences in the expression of tRNA molecules with the appropriate anticodon sequence. That is, one factor in the species-specific **codon preference** is the relationship between a codon and the amount of corresponding anticodon tRNA expressed.

Detailed Description Text - DETX (20):

There are 64 possible combinations of the 4-DNA nucleotides in codon groups of 3, and the genetic code is redundant for many of the 20 amino acids. Each of the different codons for a given amino acid encodes the incorporation of that amino acid into a polypeptide. However, within a given species there tends to be a preference for certain of the redundant codons to encode a given amino acid. The "**codon preference**" of R. mulleri is different from that of humans (this **codon preference** is usually based upon differences in the level of expression of the tRNAs containing the corresponding anticodon sequences). Table 1 shows the preferred codons for human gene expression. A codon sequence is preferred for human expression if it occurs to the left of a given codon sequence in the table. Optimally, but not necessarily, less preferred codons in a non-human polynucleotide coding **sequence are humanized by altering them to the codon most preferred for that amino acid in human gene** expression.

Detailed Description Text - DETX (80):

B. Use of humanized polynucleotides encoding R. mulleri for analysis of transcriptional regulatory sequences.

Detailed Description Text - DETX (81):

The humanized R. mulleri GFP genes of the invention allow a range of transcriptional regulatory sequences to be tested for their suitability for use with a given gene, cell, or system, but preferably for use with mammalian cells, preferably human cells. This applies to in vitro uses, such as in identifying a suitable transcriptional regulatory sequence for use in recombinant expression and high level protein production, as well as in vivo uses, such as in pre-clinical testing or in gene therapy in human subjects.

Detailed Description Text - DETX (84):

The humanized GFP encoded by a regulatory sequence testing construct may optionally have a secretion signal fused to it, such that GFP secreted to the medium is detected.

Detailed Description Text - DETX (87):

Humanized polynucleotide sequences encoding R. mulleri GFP are useful in screening assays to detect compounds that modulate transcription. In this aspect of the invention, humanized R. mulleri GFP coding sequences are positioned downstream of a promoter that is known to be inducible by the agent that one wishes to detect. Expression of GFP in the cells will normally be silent, and is activated by exposing the cell to a composition that contains the selected agent. In using a promoter that is responsive to, for example, a lipid soluble transcriptional modulator, a toxin, a hormone, a cytokine, a growth factor or other defined molecule, the presence the particular defined molecule can be determined. For example, an estrogen-responsive regulatory sequence may be linked to GFP in order to test for the presence of estrogen in a sample.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	32075	(humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1)	USPAT; US-PGPUB	2003/12/16 11:41
2	L2	8	1 same (regulat\$8 and codon adj preference)	USPAT; US-PGPUB	2003/12/16 11:42
3	L3	244	(transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon adj preference	USPAT; US-PGPUB	2003/12/16 11:54
4	L4	175	3 and 1	USPAT; US-PGPUB	2003/12/16 11:55

PGPUB-DOCUMENT-NUMBER: 20030226173

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030226173 A1

TITLE: Yield-related polynucleotides and polypeptides in  
plants

PUBLICATION-DATE: December 4, 2003

US-CL-CURRENT: 800/281, 800/284

APPL-NO: 10/ 225066

DATE FILED: August 9, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60310847 20010809 US

non-provisional-of-provisional 60336049 20011119 US

non-provisional-of-provisional 60338692 20011211 US

[0001] This application claims the benefit of U.S. Non-provisional Application No. Ser. 09/837,444, filed Apr. 18, 2001, U.S. Provisional Application No. 60/310,847, filed Aug. 9, 2001, U.S. Provisional Application No. 60/336,049, filed Dec. 5, 2001, U.S. Provisional Application No. 60/338,692, filed Dec. 11, 2001, and U.S. Non-provisional Application Ser. No. 10/171,468, filed Jun. 14, 2002, the entire contents of which are hereby incorporated by reference.

PGPUB-DOCUMENT-NUMBER: 20030217383

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030217383 A1

TITLE: Stress-related polynucleotides and polypeptides in  
plants

PUBLICATION-DATE: November 20, 2003

US-CL-CURRENT: 800/279, 435/468 , 536/23.6

APPL-NO: 10/ 225068

DATE FILED: August 9, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60310847 20010809 US

non-provisional-of-provisional 60336049 20011119 US

non-provisional-of-provisional 60338692 20011211 US

[0001] This application claims the benefit of U.S. Non-provisional application Ser. No. 09/837,444, filed Apr. 18, 2001, U.S. Provisional Application No. 60/310,847, filed Aug. 9, 2001, U.S. Provisional Application No. 60/336,049, filed Dec. 5, 2001, U.S. Provisional Application No. 60/338,692, filed Dec. 11, 2001, and U.S. Non-provisional application Ser. No. 10/171,468, filed Jun. 14, 2002, the entire contents of which are hereby incorporated by reference.

PGPUB-DOCUMENT-NUMBER: 20030211510

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030211510 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

PUBLICATION-DATE: November 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Henderson, Robert A.	Edmonds	WA	US	
Wang, Tongtong	Medina	WA	US	
Watanabe, Yoshihiro	Mercer Island	WA	US	
Kalos, Michael D.	Seattle	WA	US	
Sleath, Paul R.	Seattle	WA	US	
Johnson, Jeffrey C.	Des Moines	WA	US	
Retter, Marc W.	Carnation	WA	US	
Durham, Margarita	Seattle	WA	US	
Carter, Darrick	Seattle	WA	US	
Fanger, Gary R.	Mill Creek	WA	US	
Vedvick, Thomas S.	Federal Way	WA	US	
Bangur, Chaitanya S.	Seattle	WA	US	
McNabb, Andria	Renton	WA	US	

APPL-NO: 10/ 283017

DATE FILED: October 28, 2002

RELATED-US-APPL-DATA:

child 10283017 A1 20021028

parent continuation-in-part-of 10113872 20020328 US PENDING

child 10113872 20020328 US

parent continuation-in-part-of 10017754 20011029 US PENDING

child 10017754 20011029 US

parent continuation-in-part-of 09902941 20010710 US PENDING

child 09902941 20010710 US

parent continuation-in-part-of 09849626 20010503 US PENDING

child 09849626 20010503 US

parent continuation-in-part-of 09736457 20001213 US GRANTED  
parent-patent 6509448 US  
child 09736457 20001213 US  
parent continuation-in-part-of 09702705 20001030 US GRANTED  
parent-patent 6504010 US  
child 09702705 20001030 US  
parent continuation-in-part-of 09677419 20001006 US PENDING  
child 09677419 20001006 US  
parent continuation-in-part-of 09671325 20000926 US PENDING  
child 09671325 20000926 US  
parent continuation-in-part-of 09658824 20000908 US PENDING  
child 09658824 20000908 US  
parent continuation-in-part-of 09651563 20000829 US PENDING  
child 09651563 20000829 US  
parent continuation-in-part-of 09614124 20000711 US PENDING  
child 09614124 20000711 US  
parent continuation-in-part-of 09589184 20000605 US PENDING  
child 09589184 20000605 US  
parent continuation-in-part-of 09560406 20000427 US PENDING  
child 09560406 20000427 US  
parent continuation-in-part-of 09546259 20000410 US ABANDONED  
child 09546259 20000410 US  
parent continuation-in-part-of 09533077 20000322 US ABANDONED  
child 09533077 20000322 US  
parent continuation-in-part-of 09519642 20000306 US PENDING  
child 09519642 20000306 US  
parent continuation-in-part-of 09476300 19991230 US PENDING

child 09476300 19991230 US

parent continuation-in-part-of 09466867 19991217 US PENDING

child 09466867 19991217 US

parent continuation-in-part-of 09419356 19991015 US ABANDONED

child 09419356 19991015 US

parent continuation-in-part-of 09346492 19990630 US ABANDONED

US-CL-CURRENT: 435/6, 424/93.7 , 435/320.1 , 435/372 , 435/69.1 , 435/7.23  
, 530/350

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

----- KWIC -----

#### Summary of Invention Paragraph - BSTX (1193):

[1188] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

#### Detail Description Paragraph - DETX (94):

[1414] To facilitate the generation, purification, and evaluation of MAb against L985P, MAbs against the entire deduced amino acid sequence of the L985P protein, peptides derived from L985P or chemically produced (synthetic) L985P peptides will be used. Also, one can use MAbs raised against chimeric forms of L985P protein molecule fused to Ra12 protein, either the long form (Ra12- which is the first 128 amino acids of Ra12) and/or the short form (Ra12S), or fused to a polyhistidine peptide or any combination of these molecules. Provided are the predicted cDNA and amino acid sequences for the his-tagged L985P-Ra12 fusion molecules: Ra12-L985P\_cDNA (SEQ ID NO:1875), Ra12-L985P\_Protein (SEQ ID



NO:1876), Ra12S-L985P\_cDNA (SEQ ID NO:1877) and Ra12S-L985P\_Protein (SEQ ID NO:1878); and the L985P derived peptides: his-tagged Ra12S-L985PEx-cDNA (SEQ ID NO:1879), his-tagged Ra12S-L985PEx\_Protein (SEQ ID NO:1880), L985P-Extracellular-Loop-2 cDNA (SEQ ID NO:1881) and L985P\_Extracellular-Loop-2-Peptide (SEQ ID NO:1882).

PGPUB-DOCUMENT-NUMBER: 20030206918

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030206918 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of ovarian cancer

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fanger, Gary R.	Mill Creek	WA	US	
Fling, Steven P.	Bainbridge Island	WA	US	

APPL-NO: 10/ 361811

DATE FILED: February 5, 2003

RELATED-US-APPL-DATA:

child 10361811 A1 20030205

parent continuation-in-part-of 10212677 20020802 US PENDING

child 10212677 20020802 US

parent continuation-in-part-of 09970966 20011002 US PENDING

child 09970966 20011002 US

parent continuation-in-part-of 09825294 20010403 US PENDING

child 09825294 20010403 US

parent continuation-in-part-of 09713550 20001114 US GRANTED

parent-patent 6617109 US

child 09713550 20001114 US

parent continuation-in-part-of 09656668 20000907 US PENDING

child 09656668 20000907 US

parent continuation-in-part-of 09640173 20000815 US GRANTED

parent-patent 6613515 US

child 09640173 20000815 US

parent continuation-in-part-of 09561778 20000501 US ABANDONED

child 09561778 20000501 US

parent continuation-in-part-of 09394374 19990910 US ABANDONED

US-CL-CURRENT: 424/185.1, 435/320.1 , 435/325 , 435/69.3 , 530/350  
, 536/23.5

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

----- KWIC -----

Summary of Invention Paragraph - BSTX (248):

[0244] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030194764

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030194764 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

PUBLICATION-DATE: October 16, 2003

US-CL-CURRENT: 435/69.1, 435/183, 435/320.1, 435/325, 530/350, 536/23.1

APPL-NO: 10/ 116712

DATE FILED: April 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60327511 20011005 US

non-provisional-of-provisional 60282289 20010405 US

PGPUB-DOCUMENT-NUMBER: 20030190697

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190697 A1

TITLE: Novel co-stimulatory molecules

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Punnonen, Juha	Belmont	CA	US	
Lazetic, Alexandra L.L.	San Jose	CA	US	
Leong, Steven R.	Berkeley	CA	US	
Chang, Chia-Chun Jean	Los Gatos	CA	US	
Apt, Doris	Sunnyvale	CA	US	
Gustafsson, Claes	Belmont	CA	US	

APPL-NO: 09/ 888324

DATE FILED: June 22, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60213946 20000623 US

non-provisional-of-provisional 60241245 20001017 US

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 530/350 , 536/23.5

ABSTRACT:

The invention provides polynucleotides and polypeptides encoded therefrom having advantageous properties, including an ability of the polypeptides to preferentially bind a CD28 or CTLA-4 receptor at a level greater or less than the ability of human B7-1 to bind CD28 or CTLA-4, or to induce or inhibit altered level of T cell proliferation response greater compared to that generated by human B7-1. The polypeptides and polynucleotides of the invention are useful in therapeutic and prophylactic treatment methods, gene therapy applications, and vaccines.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Provisional Patent Application Serial Nos. 60/213,946, filed on Jun. 23, 2000, and 60/241,245, filed on Oct. 17, 2000, the disclosure of each of which is incorporated herein by reference in its entirety for all purposes.

----- KWIC -----

Detail Description Paragraph - DETX (11):

[0097] A nucleic acid, protein, peptide, polypeptide, or other component is "isolated" when it is partially or completely separated from components with which it is normally associated (other peptides, polypeptides, proteins (including complexes, e.g., polymerases and ribosomes which may accompany a native sequence), nucleic acids, cells, synthetic reagents, cellular contaminants, cellular components, etc.), e.g., such as from other components with which it is normally associated in the cell from which it was originally derived. A nucleic acid, polypeptide, or other component is isolated when it is partially or completely recovered or separated from other components of its natural environment such that it is the predominant species present in a composition, mixture, or collection of components (i.e., on a molar basis it is more abundant than any other individual species in the composition). In preferred embodiments, the preparation consists of more than about 70% or 75%, typically more than about 80%, or preferably more than about 90% of the isolated species.

Detail Description Paragraph - DETX (108):

[0194] The polynucleotide sequences of the present invention can be engineered in order to alter an NCSM coding sequence of the invention for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc. Further details regarding silent and conservative substitutions are provided below.

Detail Description Paragraph - DETX (114):

[0200] The polynucleotides of the present invention and fragments and variants thereof, which encode the NCSM polypeptide molecules, may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40, bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adeno-associated virus, retroviruses and many others. Any vector that transduces genetic material into a cell, and, if replication is desired, which is replicable and viable in the relevant host can be used.

Detail Description Paragraph - DETX (249):

[0332] Oligonucleotide directed mutagenesis can be used to introduce site-specific mutations in a nucleic acid sequence of interest. Examples of such techniques are found in the references above and, e.g., in Reidhaar-Olson et al. (1988) Science, 241:53-57. Similarly, cassette mutagenesis can be used in a process that replaces a small region of a double stranded DNA molecule with a synthetic oligonucleotide cassette that differs from the native sequence. The oligonucleotide can contain, e.g., completely and/or partially

randomized native sequence(s).

Detail Description Paragraph - DETX (344):

[0427] In order to produce antisera for use in an immunoassay, one or more of the immunogenic polypeptides is produced and purified as described herein. For example, recombinant protein may be produced in a mammalian cell line. An inbred strain of mice (used in this assay because results are more reproducible due to the virtual genetic identity of the mice) is immunized with the immunogenic protein(s) in combination with a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, supra, for a standard description of antibody generation, immunoassay formats and conditions that can be used to determine specific immunoreactivity). Alternatively, one or more synthetic or recombinant polypeptides derived from the sequences disclosed herein is conjugated to a carrier protein and used as an immunogen.

PGPUB-DOCUMENT-NUMBER: 20030188330

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030188330 A1

TITLE: Genes for modifying plant traits xi

PUBLICATION-DATE: October 2, 2003

US-CL-CURRENT: 800/278, 530/370 , 536/23.6

APPL-NO: 10/ 112887

DATE FILED: March 18, 2002



PGPUB-DOCUMENT-NUMBER: 20030185830

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030185830 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of prostate cancer

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Xu, Jiangchun	Bellevue	WA	US	
Stolk, John A.	Bothell	WA	US	
Kalos, Michael D.	Seattle	WA	US	

APPL-NO: 10/ 294025

DATE FILED: November 12, 2002

RELATED-US-APPL-DATA:

child 10294025 A1 20021112

parent continuation-in-part-of 10144678 20020509 US PENDING

child 10144678 20020509 US

parent continuation-in-part-of 10012896 20011210 US PENDING

child 10012896 20011210 US

parent continuation-in-part-of 09895814 20010629 US PENDING

child 09895814 20010629 US

parent continuation-in-part-of 09852911 20010509 US ABANDONED

child 09852911 20010509 US

parent continuation-in-part-of 09780669 20010209 US PENDING

child 09780669 20010209 US

parent continuation-in-part-of 09759143 20010112 US PENDING

child 09759143 20010112 US

parent continuation-in-part-of 09709729 20001109 US ABANDONED

child 09709729 20001109 US  
parent continuation-in-part-of 09685166 20001010 US PENDING  
child 09685166 20001010 US  
parent continuation-in-part-of 09679426 20001002 US PENDING  
child 09679426 20001002 US  
parent continuation-in-part-of 09657279 20000906 US PENDING  
child 09657279 20000906 US  
parent continuation-in-part-of 09651236 20000829 US PENDING  
child 09651236 20000829 US  
parent continuation-in-part-of 09636215 20000809 US PENDING  
child 09636215 20000809 US  
parent continuation-in-part-of 09605783 20000627 US PENDING  
child 09605783 20000627 US  
parent continuation-in-part-of 09593793 20000613 US PENDING  
child 09593793 20000613 US  
parent continuation-in-part-of 09570737 20000512 US PENDING  
child 09570737 20000512 US  
parent continuation-in-part-of 09568100 20000509 US PENDING  
child 09568100 20000509 US  
parent continuation-in-part-of 09536857 20000327 US ABANDONED  
child 09536857 20000327 US  
parent continuation-in-part-of 09483672 20000114 US PENDING  
child 09483672 20000114 US  
parent continuation-in-part-of 09443686 19991118 US ABANDONED  
child 09443686 19991118 US  
parent continuation-in-part-of 09439313 19991112 US GRANTED  
parent-patent 6329505 US

child 09439313 19991112 US

parent continuation-in-part-of 09352616 19990713 US GRANTED

parent-patent 6395278 US

child 09352616 19990713 US

parent continuation-in-part-of 09288946 19990409 US ABANDONED

child 09288946 19990409 US

parent continuation-in-part-of 09232149 19990115 US GRANTED

parent-patent 6465611 US

child 09232149 19990115 US

parent continuation-in-part-of 09159812 19980923 US PENDING

child 09159812 19980923 US

parent continuation-in-part-of 09115453 19980714 US PENDING

child 09115453 19980714 US

parent continuation-in-part-of 09030607 19980225 US GRANTED

parent-patent 6262245 US

child 09030607 19980225 US

parent continuation-in-part-of 09020956 19980209 US GRANTED

parent-patent 6261562 US

child 09020956 19980209 US

parent continuation-in-part-of 08904804 19970801 US ABANDONED

child 08904804 19970801 US

parent continuation-in-part-of 08806099 19970225 US ABANDONED

US-CL-CURRENT: 424/155.1, 424/93.21, 435/320.1, 435/325, 435/372, 435/6  
, 435/69.3, 435/7.23, 530/350, 536/23.2

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly prostate cancer, are disclosed. Illustrative compositions comprise one or more prostate-specific polypeptides, immunogenic portions thereof, polynucleotides

that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly prostate cancer.

----- KWIC -----

Detail Description Paragraph - DETX (109):

[0836] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030175700

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175700 A1

TITLE: Compounds and methods for treatment and diagnosis of  
chlamydial infection

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bhatia, Ajay	Seattle	WA	US	
Probst, Peter	Seattle	WA	US	
Stromberg, Erika Jean	Seattle	WA	US	

APPL-NO: 09/ 841260

DATE FILED: April 23, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60198853 20000421 US

non-provisional-of-provisional 60219752 20000720 US

US-CL-CURRENT: 435/6, 424/190.1, 435/183, 435/252.3, 435/320.1, 435/69.3  
, 435/7.36, 530/350, 536/23.7

ABSTRACT:

Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a Chlamydia antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 60/198,853, filed Apr. 21, 2000, and U.S. Provisional Application No. 60/219,752, filed Jul. 20, 2000, incorporated in their entirety herein.

----- KWIC -----

Summary of Invention Paragraph - BSTX (197):

[0194] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX (281):

[0278] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20030175294

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175294 A1

TITLE: Heterologous fusion protein constructs comprising a  
Leishmania antigen

PUBLICATION-DATE: September 18, 2003

US-CL-CURRENT: 424/190.1, 424/191.1, 435/189, 435/252.3, 435/320.1  
, 435/69.3, 536/23.7

APPL-NO: 10/ 098732

DATE FILED: March 13, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60275837 20010313 US

#### CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Ser. No. 60/275,837, filed Mar. 13, 2001, herein incorporated by reference in its entirety.

[0002] The present application is related to U.S. patent application Ser. No. 09/056,556, filed Apr. 7, 1998; U.S. patent application Ser. No. 09/223,040, filed Dec. 30, 1998; U.S. patent application Ser. No. 09/287,849, filed Apr. 7, 1999; published PCT application No. WO 99/51748, filed Apr. 7, 1999 (PCT/US99/07717); U.S. patent application Ser. No. 60/158,338, filed Oct. 7, 1999; U.S. patent application Ser. No. 60/158,425, filed Oct. 7, 1999; U.S. patent application Ser. No. 09/597,796, filed Jun. 20, 2000; U.S. patent application Ser. No. 09/688,672, filed Oct. 10, 2000; published PCT application No. WO 01/24820, filed Oct. 10, 2000 (PCT/US00/28095); U.S. patent application Ser. No. 60/265,737, filed Feb. 1, 2001; U.S. patent application Ser. No. 09/886,349, filed Jun. 20, 2001; and published PCT application No. WO 01/98460, filed Jun. 20, 2001 (PCT/US01 19959), and U.S. Ser. No. 60/\_\_\_\_\_, filed Feb. 15, 2002, TTC attorney docket number 014058-009080US, herein each incorporated by reference in its entirety.

PGPUB-DOCUMENT-NUMBER: 20030170255

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030170255 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Watanabe, Yoshihiro	Mercer Island	WA	US	
Henderson, Robert A.	Edmonds	WA	US	
Kalos, Michael D.	Seattle	WA	US	
Sleath, Paul R.	Seattle	WA	US	
Vedvick, Thomas S.	Federal Way	WA	US	
Carter, Darrick	Seattle	WA	US	
Fanger, Gary R.	Mill Creek	WA	US	

APPL-NO: 10/ 113872

DATE FILED: March 28, 2002

RELATED-US-APPL-DATA:

child 10113872 A1 20020328

parent continuation-in-part-of 10017754 20011029 US PENDING

child 10017754 20011029 US

parent continuation-in-part-of 09902941 20010710 US PENDING

child 09902941 20010710 US

parent continuation-in-part-of 09849626 20010503 US PENDING

child 09849626 20010503 US

parent continuation-in-part-of 09736457 20001213 US GRANTED

parent-patent 6509448 US

child 09736457 20001213 US

parent continuation-in-part-of 09702705 20001030 US GRANTED

parent-patent 6504010 US



child 09702705 20001030 US  
parent continuation-in-part-of 09677419 20001006 US PENDING  
child 09677419 20001006 US  
parent continuation-in-part-of 09671325 20000926 US PENDING  
child 09671325 20000926 US  
parent continuation-in-part-of 09658824 20000908 US PENDING  
child 09658824 20000908 US  
parent continuation-in-part-of 09651563 20000829 US PENDING  
child 09651563 20000829 US  
parent continuation-in-part-of 09614124 20000711 US PENDING  
child 09614124 20000711 US  
parent continuation-in-part-of 09589184 20000605 US PENDING  
child 09589184 20000605 US  
parent continuation-in-part-of 09560406 20000427 US PENDING  
child 09560406 20000427 US  
parent continuation-in-part-of 09546259 20000410 US ABANDONED  
child 09546259 20000410 US  
parent continuation-in-part-of 09533077 20000322 US ABANDONED  
child 09533077 20000322 US  
parent continuation-in-part-of 09519642 20000306 US PENDING  
child 09519642 20000306 US  
parent continuation-in-part-of 09476300 19991230 US PENDING  
child 09476300 19991230 US  
parent continuation-in-part-of 09466867 19991217 US PENDING  
child 09466867 19991217 US  
parent continuation-in-part-of 09419356 19991015 US ABANDONED  
child 09419356 19991015 US

parent continuation-in-part-of 09346492 19990630 US ABANDONED

US-CL-CURRENT: 424/185.1

**ABSTRACT:**

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 10/017,754, filed Oct. 29, 2001, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/902,941, filed Jul. 10, 2001, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/849,626, filed May 3, 2001, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/736,457, filed Dec. 13, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/702,705, filed Oct. 30, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/677,419, filed Oct. 6, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/671,325, filed Sep. 26, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/658,824, filed Sep. 8, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/651,563, filed Aug. 29, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/614,124, filed Jul. 11, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/589,184, filed Jun. 5, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/560,406, filed Apr. 27, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/546,259, filed Apr. 10, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/533,077, filed Mar. 22, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/519,642, filed Mar. 6, 2000, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/476,300, filed Dec. 30, 1999, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/466,867, filed Dec. 17, 1999, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/419,356, filed Oct. 15, 1999, now pending; which is a continuation-in-part of U.S. patent application Ser. No. 09/346,492, filed Jun. 30, 1999, now pending, which applications are incorporated herein by reference in their entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX (1178):

[1174] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX (94):

[1401] To facilitate the generation, purification, and evaluation of MAb against L985P, MAbs against the entire deduced amino acid sequence of the L985P protein, peptides derived from L985P or chemically produced (synthetic) L985P peptides will be used. Also, one can use MAbs raised against chimeric forms of L985P protein molecule fused to Ra12 protein, either the long form (Ra12--which is the first 128 amino acids of Ra12) and/or the short form (Ra12S), or fused to a polyhistidine peptide or any combination of these molecules. Provided are the predicted cDNA and amino acid sequences for the his-tagged L985P-Ra12 fusion molecules: Ra12-L985P\_cDNA (SEQ ID NO:1875), Ra12-L985P\_Protein (SEQ ID NO:1876), Ra12S-L985P\_cDNA (SEQ ID NO:1877) and Ra12S-L985P\_Protein (SEQ ID NO:1878); and the L985P derived peptides: his-tagged Ra12S-L985PEx\_cDNA (SEQ ID NO:1879), his-tagged Ra12S-L985PEx\_Protein (SEQ ID NO:1880), L985P\_Extracellular\_Loop-2\_cDNA (SEQ ID NO:1881) and L985P\_Extracellular\_Loop-2\_Peptide (SEQ ID NO:1882).

PGPUB-DOCUMENT-NUMBER: 20030167537

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030167537 A1

TITLE: Method for modifying plant biomass

PUBLICATION-DATE: September 4, 2003

US-CL-CURRENT: 800/290, 435/410, 435/6, 536/23.2, 536/23.6, 800/278

APPL-NO: 09/ 823676

DATE FILED: March 30, 2001

PGPUB-DOCUMENT-NUMBER: 20030167531

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030167531 A1

TITLE: Expression and purification of bioactive, authentic polypeptides from plants

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Russell, Douglas A.	Madison	WI	US	
Schlittler, Michael	Wildwood	MO	US	

APPL-NO: 09/ 824200

DATE FILED: April 3, 2001

RELATED-US-APPL-DATA:

child 09824200 A1 20010403

parent continuation-in-part-of 09113244 19980710 US GRANTED

parent-patent 6512162 US

child 09824200 A1 20010403

parent continuation-in-part-of 09316847 19990521 US ABANDONED

non-provisional-of-provisional 60194217 20000403 US

US-CL-CURRENT: 800/288, 530/351

ABSTRACT:

The present invention relates to a process for the production of proteins or polypeptides using genetically manipulated plants or plant cells, as well as to the genetically manipulated plants and plant cells per se (including parts of the genetically manipulated plants), the heterologous protein material (e.g., a protein, polypeptide and the like) which is produced with the aid of these genetically manipulated plants or plant cells, and the recombinant polynucleotides (DNA or RNA) that are used for the genetic manipulation.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present invention is related to and claims the benefit, under 35 U.S.C. .sectn.120, of patent applications Ser. Nos. 09/113,244, filed Jul. 10, 1998, U.S. Ser. No. 09/316,847, filed May 20, 1999, and is related to and

claims the benefit, under 35 U.S.C. .sectn.119(e), of provisional patent application Serial No. 60/194,217, filed Apr. 3, 2000, which are expressly incorporated fully herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX (18):

[0075] In accordance with one aspect of the present invention, methods and materials are provided for a novel means of production of recombinant cytokines in a plant host system that are easily separated from other host cell compartments. Purification of the recombinant cytokine is greatly simplified by this approach. The recombinant nucleic acid encoding the cytokine may be part of all of a naturally occurring DNA sequence from any source, it may be a synthetic DNA sequence or it may be a combination of naturally occurring and synthetic sequences. The present invention includes the steps, singly or in sequence, of preparing an expression vector that includes a first nucleic acid sequence that regulates the transcription of a second nucleic acid sequence encoding a significant portion of a peptide that targets a protein to a sub-cellular location, and, fused to this second nucleic acid, a third nucleic acid encoding the cytokine of interest; generating a transformed plant host system in which the cytokine of interest is expressed; and purifying the cytokine of interest from the transgenic plant host system.

Detail Description Paragraph - DETX (26):

[0082] Amino acid sequences: as used herein, includes an oligopeptide, peptide, polypeptide, or protein sequence, and fragment thereof, and to naturally occurring or synthetic molecules.

Detail Description Paragraph - DETX (79):

[0135] Additionally, when expressing in non-human cells, the polynucleotides encoding the cytokine may be modified in the silent position of any triplet amino acid codon so as to better conform to the codon preference of the particular host organism. More specifically, translational efficiency of a protein in a given host organism can be regulated through codon bias, meaning that the available 61 codons for a total of 20 amino acids are not evenly used in translation, an observation that has been made for prokaryotes (Kane, 6 Current Op. Biotech. 494-500 (1995)), and eukaryotes (Ernst, Codon Usage & Gene Expression 196-99 (Elsevier Pub., Cambridge 1988). An application of these observations, i.e., the adaptation of the codon bias of a bacterial gene to the codon bias of a higher plant, resulted in significantly higher accumulation of the foreign protein in the plant. Perlak et al., 88(8) P.N.A.S. 3324-28 (1991); see also Murray et al., 17 Nucl. Acids Res. 477-98 (1989); U.S. Pat. No. 6,121,014. Codon usage tables have been established not only for organisms, but also for organelles and specific tissues (Kazusa DNA Research Inst., &lt;www.kazusa.or.jp>), and their general availability enables researchers to adopt the codon usage of a given gene to the host organism. Other factors like the context of the initiator methionine start codon (Kozak, 234 Gene 187-208 (1999)), may influence the translation rate of a given protein in a host organism, and can therefore be taken into

consideration. See also Taylor et al., 210 Mol. Genetics 572-77 (1987). Translation may also be optimized by reference to codon sequences that may generate potential signals of intron splice sites. Plant Mol. Bio. Labfax (Croy, ed. 1993), mRNA instability and polyadenylation signals (Perlak et al., supra).

Detail Description Paragraph - DETX (136):

[0189] The design entailed a synthetic gene that encoded soy oleosin, an enterokinase protease recognition site, and a fragment of the hGH amino terminus. This was inserted between a plant 5' UTR, and the remaining fragment of hGH, to create pmon41324. While the oleosin fusion may aid in correct folding and potential purification of hGH, the enterokinase site allows later specific protease cleavage at AspAspAspAspLys/PheProThr (SEQ ID NO:06), to yield the mature natural amino terminus of hGH. Reduced SDS-PAGE Western blot analysis of the transient soy hypocotyl extracts (FIG. 6) shows a significant increase in expression level of the correct-sized fusion product (OLE) relative to the non-fused extensin control (EXT), with very little evidence of the 14 kD truncated fragment. In FIG. 6, the left lane in each pair was from extractions with 20 mM Tris-Cl pH 7.5, 0.01% Triton X-100, 5% glycerol, and 50 mM NaCl. The right lane in each pair was from extractions with 20 mM Tris-Cl pH 7.5, 4 mM CHAPS, 5% glycerol, and 50 mM NaCl. The 1 ng hGH standard has a monomer band that co-migrated with the secreted hGH design, while the oleosin fusion migrated more slowly, as expected for a fusion.

PGPUB-DOCUMENT-NUMBER: 20030166064

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030166064 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of colon cancer

PUBLICATION-DATE: September 4, 2003

US-CL-CURRENT: 435/69.1, 536/23.1

APPL-NO: 10/ 099926

DATE FILED: March 14, 2002

RELATED-US-APPL-DATA:

child 10099926 A1 20020314

parent continuation-in-part-of 10033528 20011226 US PENDING

child 10033528 20011226 US

parent continuation-in-part-of 09920300 20010731 US PENDING

non-provisional-of-provisional 60302051 20010629 US

non-provisional-of-provisional 60279763 20010328 US

non-provisional-of-provisional 60223283 20000803 US



PGPUB-DOCUMENT-NUMBER: 20030166022

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030166022 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of breast cancer

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Houghton, Raymond L.	Bothell	WA	US	
Sleath, Paul R.	Seattle	WA	US	
Persing, David H.	Redmond	WA	US	

APPL-NO: 10/ 124805

DATE FILED: April 15, 2002

RELATED-US-APPL-DATA:

child 10124805 A1 20020415

parent continuation-in-part-of 10076622 20020213 US PENDING

child 10076622 20020213 US

parent continuation-in-part-of 10007805 20011207 US PENDING

child 10007805 20011207 US

parent continuation-in-part-of 09834759 20010413 US PENDING

child 09834759 20010413 US

parent continuation-in-part-of 09620405 20000720 US GRANTED

parent-patent 6528054 US

child 09620405 20000720 US

parent continuation-in-part-of 09604287 20000622 US GRANTED

parent-patent 6586572 US

child 09604287 20000622 US

parent continuation-in-part-of 09590751 20000608 US PENDING

child 09590751 20000608 US

parent continuation-in-part-of 09551621 20000417 US PENDING

child 09551621 20000417 US

parent continuation-in-part-of 09433826 19991103 US GRANTED

parent-patent 6579973 US

child 09433826 19991103 US

parent continuation-in-part-of 09389681 19990902 US GRANTED

parent-patent 6518237 US

child 09389681 19990902 US

parent continuation-in-part-of 09339338 19990623 US GRANTED

parent-patent 6573368 US

child 09339338 19990623 US

parent continuation-in-part-of 09285480 19990402 US GRANTED

parent-patent 6590076 US

child 09285480 19990402 US

parent continuation-in-part-of 09222575 19981228 US GRANTED

parent-patent 6387697 US

US-CL-CURRENT: 435/7.23, 435/183 , 435/320.1 , 435/325 , 435/69.1  
 , 530/388.8 , 536/23.2

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

----- KWIC -----

Detail Description Paragraph - DETX (686):

[0731] Moreover, the polynucleotide sequences of the present invention can

be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX (§35):

[0964] Thirty-one overlapping synthetic peptides spanning the entire B726P downstream ORF sequence (amino acid sequence set forth in SEQ ID NO: 176) were synthesized and 30 of these were tested in ELISA with sera from breast cancer patients as well as control sera. The amino acid sequences of the 31 overlapping peptides of the B726P downstream ORF are set forth in SEQ ID NO: 594-624. Three additional peptides of B726P, set forth in SEQ ID NO: 625-627 were also tested. Several peptides throughout the molecule showed reactivity, in particular peptide #2735 (amino acids 31-50; SEQ ID NO: 597), peptide #2747 (amino acids 151-170; SEQ ID NO: 609), peptide #2750 (amino acids 181-200; SEQ ID NO: 612), peptide #2753 (amino acids 211-230; SEQ ID NO: 615), and peptide #2766 (amino acids 231-250; SEQ ID NO: 617). A total of [fraction (16/74)] breast cancer sera were reactive with at least one peptide.

PGPUB-DOCUMENT-NUMBER: 20030165820

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030165820 A1

TITLE: Compositions and methods for the diagnosis and  
treatment of herpes simplex virus infection

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Day, Craig H.	Shoreline	WA	US	
Hosken, Nancy A.	Seattle	WA	US	
Parsons, Joseph M.	Seattle	WA	US	

APPL-NO: 10/ 237551

DATE FILED: September 6, 2002

RELATED-US-APPL-DATA:

child 10237551 A1 20020906

parent continuation-in-part-of 10200562 20020719 US PENDING

child 10200562 20020719 US

parent continuation-in-part-of 10121988 20020411 US PENDING

child 10121988 20020411 US

parent continuation-in-part-of 09894998 20010628 US PENDING

non-provisional-of-provisional 60277438 20010320 US

non-provisional-of-provisional 60215458 20000629 US

US-CL-CURRENT: 435/5, 424/186.1, 435/235.1, 435/320.1, 435/366, 435/69.3  
, 514/44, 530/350, 536/23.72

ABSTRACT:

Compounds and methods for the diagnosis and treatment of HSV infection are provided. The compounds comprise polypeptides that contain at least one antigenic portion of an HSV polypeptide and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits are also provided

comprising such polypeptides and/or DNA sequences and a suitable detection reagent for the detection of HSV infection in patients and in biological samples.

----- KWIC -----

#### Summary of Invention Paragraph - BSTX (314):

[0310] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

#### Summary of Invention Paragraph - BSTX (398):

[0394] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

#### Detail Description Paragraph - DETX (230):

[0750] CD8.sup.+ T cells isolated from a panel of HSV-2 seropositive donors were screened for their ability to respond to a variety of HSV-2 proteins. Briefly, PBMCs were obtained from donors EB5491, AG10295, LM10295, and 447, and enriched for CD8.sup.+ T cells using microbeads or CD8+ Enrichment Kits from Miltenyi. Synthetic peptides (15 amino acids in length and overlapping in sequence by 10 or 11 amino acids) were synthesized across several complete or partial ORFs from HSV-2 strain HG52, including proteins UL21 (the full length DNA/amino acids of which are disclosed in SEQ ID NOS.:144 and 154, respectively), UL50 (the full length DNA/amino acids of which are disclosed in SEQ ID NOS.:145 and 153, respectively), US3 (the full length DNA/amino acids of which are disclosed in SEQ ID NOS.:146 and 154, respectively), UL54 (the full length DNA/amino acids of which are disclosed in SEQ ID NOS.:147 and 156, respectively), US8 (the full length DNA/amino acids of which are disclosed in SEQ ID NOS.:148 and 157, respectively), UL19 (the full length DNA/amino acids of which are disclosed in SEQ ID NOS.:149 and 158, respectively), UL46 (the full length DNA/amino acids of which are disclosed in SEQ ID NOS.:150 and 159, respectively), UL18 (the full length DNA/amino acids of which are disclosed in SEQ ID NOS.:151 and 160, respectively), and RL2 (the full length DNA/amino

acids of which are disclosed in SEQ ID NOs: 152 and 161, respectively). The peptides were screened by 24 co-culture of the donor's CD8<sup>+</sup> T cells (2-5.times.10.sup.5 cells/well), autologous dendritic cells (2-5.times.10.sup.4 cells/well) and peptides (0.5 .mu.g/ml each) in 96-well ELISPOT plates that had been pre-coated with anti-human IFN- $\gamma$  antibody. Each peptide pool was screened in an individual well. The ELISPOT plates were developed as per a standard protocol. The number of spots per well was counted using an automated video-microscopy ELISPOT reader. Individual 15-mer peptides, determined from peptide pools testing positive, were screened as described above and returned the following results:

PGPUB-DOCUMENT-NUMBER: 20030165819

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030165819 A1

TITLE: Compositions and methods for the diagnosis and  
treatment of herpes simplex virus infection

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
McGowan, Patrick	Seattle	WA	US	
Hosken, Nancy A.	Seattle	WA	US	

APPL-NO: 10/ 200562

DATE FILED: July 19, 2002

RELATED-US-APPL-DATA:

child 10200562 A1 20020719

parent continuation-in-part-of 10121988 20020411 US PENDING

child 10121988 20020411 US

parent continuation-in-part-of 09894998 20010628 US PENDING

non-provisional-of-provisional 60277438 20010320 US

non-provisional-of-provisional 60215458 20000629 US

US-CL-CURRENT: 435/5, 435/320.1 , 435/325 , 435/69.3 , 530/350 , 536/23.72

ABSTRACT:

Compounds and methods for the diagnosis and treatment of HSV infection are provided. The compounds comprise polypeptides that contain at least one antigenic portion of an HSV polypeptide and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits are also provided comprising such polypeptides and/or DNA sequences and a suitable detection reagent for the detection of HSV infection in patients and in biological samples.

----- KWIC -----

Summary of Invention Paragraph - BSTX (272):

[0268] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX (356):

[0352] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

Detail Description Paragraph - DETX (226):

[0703] CD8<sup>sup</sup>.+ T cells isolated from a panel of HSV-2 seropositive donors were screened for their ability to respond to a variety of HSV-2 proteins. Briefly, PBMCs were obtained from donors EB5491, AG10295, LM10295, and 447, and enriched for CD8+ T cells using microbeads or CD8+Enrichment Kits from Miltenyi. Synthetic peptides (15 amino acids in length and overlapping in sequence by 10 or 11 amino acids) were synthesized across several complete or partial ORFs from HSV-2 strain HG52, including proteins UL21 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 144 and 154, respectively), UL50 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 145 and 153, respectively), US3 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 146 and 154, respectively), UL54 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 147 and 156, respectively), US8 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 148 and 157, respectively), UL19 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 149 and 158, respectively), UL46 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 150 and 159, respectively), UL18 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 151 and 160, respectively), and RL2 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 152 and 161, respectively). The peptides were screened by 24 co-culture of the donor's CD8+ T cells (2-5.times.10<sup>sup</sup>.5 cells/well), autologous dendritic cells (2-5.times.10<sup>sup</sup>.4 cells/well) and peptides (0.5 .mu.g/ml each) in 96-well ELISPOT plates that had been pre-coated with anti-human IFN- $\gamma$  antibody. Each peptide pool was screened in an individual well. The ELISPOT plates were developed as per a standard protocol. The number of spots per well was counted



using an automated video-microscopy ELISPOT reader. Individual 15-mer peptides, determined from peptide pools testing positive, were screened as described above and returned the following results:

PGPUB-DOCUMENT-NUMBER: 20030157089

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030157089 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of prostate cancer

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Xu, Jiangchun	Bellevue	WA	US	
Dillon, Davin C.	Issaquah	WA	US	
Mitcham, Jennifer L.	Redmond	WA	US	
Harlocker, Susan L.	Seattle	WA	US	
Jiang, Yuqiu	Kent	WA	US	
Henderson, Robert A.	Edmonds	WA	US	
Kalos, Michael D.	Seattle	WA	US	
Fanger, Gary R.	Mill Creek	WA	US	
Retter, Marc W.	Carnation	WA	US	
Stolk, John A.	Bothell	WA	US	
Day, Craig H.	Shoreline	WA	US	
Vedvick, Thomas S.	Federal Way	WA	US	
Carter, Darrick	Seattle	WA	US	
Li, Samuel X.	Redmond	WA	US	
Wang, Aijun	Issaquah	WA	US	
Skeiky, Yasir A.W.	Bellevue	WA	US	
Hepler, William T.	Seattle	WA	US	
Hural, John	Bainbridge Island	WA	US	
McNeill, Patricia D.	Federal Way	WA	US	
Houghton, Raymond L.	Bothell	WA	US	
Vinals y de Bassols, Carlota	Rixensart	WA	BE	
Foy, Teresa M.	Federal Way	WA	US	
Watanabe, Yoshihiro	Mercer Island	WA	US	
Meagher, Madeleine Joy	Seattle	WA	US	
Deng, Ta	Edmonds		US	

APPL-NO: 10/ 144678

DATE FILED: May 9, 2002

RELATED-US-APPL-DATA:

child 10144678 A1 20020509

parent continuation-in-part-of 10012896 20011210 US PENDING

child 10012896 20011210 US

parent continuation-in-part-of 09895814 20010629 US PENDING  
child 09895814 20010629 US

parent continuation-in-part-of 09852911 20010509 US ABANDONED  
child 09852911 20010509 US

parent continuation-in-part-of 09780669 20010209 US PENDING  
child 09780669 20010209 US

parent continuation-in-part-of 09759143 20010112 US PENDING  
child 09759143 20010112 US

parent continuation-in-part-of 09709729 20001109 US ABANDONED  
child 09709729 20001109 US

parent continuation-in-part-of 09685166 20001010 US PENDING  
child 09685166 20001010 US

parent continuation-in-part-of 09679426 20001002 US PENDING  
child 09679426 20001002 US

parent continuation-in-part-of 09657279 20000906 US PENDING  
child 09657279 20000906 US

parent continuation-in-part-of 09651236 20000829 US PENDING  
child 09651236 20000829 US

parent continuation-in-part-of 09636215 20000809 US PENDING  
child 09636215 20000809 US

parent continuation-in-part-of 09605783 20000627 US PENDING  
child 09605783 20000627 US

parent continuation-in-part-of 09593793 20000613 US PENDING  
child 09593793 20000613 US

parent continuation-in-part-of 09570737 20000512 US PENDING  
child 09570737 20000512 US

parent continuation-in-part-of 09568100 20000509 US PENDING

child 09568100 20000509 US

parent continuation-in-part-of 09536857 20000327 US ABANDONED

child 09536857 20000327 US

parent continuation-in-part-of 09483672 20000114 US PENDING

child 09483672 20000114 US

parent continuation-in-part-of 09443686 19991118 US ABANDONED

child 09443686 19991118 US

parent continuation-in-part-of 09439313 19991112 US GRANTED

parent-patent 6329505 US

child 09439313 19991112 US

parent continuation-in-part-of 09352616 19990713 US GRANTED

parent-patent 6395278 US

child 09352616 19990713 US

parent continuation-in-part-of 09288946 19990409 US ABANDONED

child 09288946 19990409 US

parent continuation-in-part-of 09232149 19990115 US GRANTED

parent-patent 6465611 US

child 09232149 19990115 US

parent continuation-in-part-of 09159812 19980923 US PENDING

child 09159812 19980923 US

parent continuation-in-part-of 09115453 19980714 US PENDING

child 09115453 19980714 US

parent continuation-in-part-of 09030607 19980225 US GRANTED

parent-patent 6262245 US

child 09030607 19980225 US

parent continuation-in-part-of 09020956 19980209 US GRANTED

parent-patent 6261562 US

child 09020956 19980209 US

parent continuation-in-part-of 08904804 19970801 US ABANDONED

child 08904804 19970801 US

parent continuation-in-part-of 08806099 19970225 US ABANDONED

US-CL-CURRENT: 424/130.1, 435/183, 435/320.1, 435/325, 435/6, 435/69.1  
, 435/7.23, 530/350, 536/23.2

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly prostate cancer, are disclosed. Illustrative compositions comprise one or more prostate-specific polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly prostate cancer.

----- KWIC -----

#### Detail Description Paragraph - DETX (779):

[0826] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030144494

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030144494 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of ovarian cancer

PUBLICATION-DATE: July 31, 2003

US-CL-CURRENT: 536/23.2, 424/155.1, 435/320.1, 435/325, 435/6, 435/7.23  
, 530/350, 530/388.8

APPL-NO: 10/ 264283

DATE FILED: October 2, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60384531 20020530 US

non-provisional-of-provisional 60327135 20011002 US

PGPUB-DOCUMENT-NUMBER: 20030138881

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138881 A1

TITLE: Novel co-stimulatory molecules

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Punnonen, Juha	Belmont	CA	US	
Lazetic, Alexandra	San Jose	CA	US	
Leong, Steven R.	Berkeley	CA	US	
Chang, Chia-Chun	Los Gatos	CA	US	
Apt, Doris	Sunnyvale	CA	US	
Gustafsson, Claes	Belmont	CA	US	

APPL-NO: 10/ 032214

DATE FILED: December 20, 2001

RELATED-US-APPL-DATA:

child 10032214 A1 20011220

parent continuation-in-part-of 09888324 20010622 US PENDING

child 10032214 A1 20011220

parent continuation-in-part-of PCT/US01/19973 20010622 US UNKNOWN

non-provisional-of-provisional 60213946 20000623 US

non-provisional-of-provisional 60241245 20001017 US

US-CL-CURRENT: 435/69.1, 435/320.1, 435/325, 530/350, 536/23.5

ABSTRACT:

The invention provides polynucleotides and polypeptides encoded therefrom having advantageous properties, including an ability of the polypeptides to preferentially bind a CD28 or CTLA-4 receptor at a level greater or less than the ability of human B7-1 to bind CD28 or CTLA-4, or to induce or inhibit altered level of T cell proliferation response greater compared to that generated by human B7-1. The polypeptides and polynucleotides of the invention are useful in therapeutic and prophylactic treatment methods, gene therapy applications, and vaccines.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part application of U.S. patent application Ser. No. 09/888,324, filed Jun. 22, 2001 and International Patent Application Serial No. PCT/US01/19973, which claim priority to and benefit of U.S. Provisional Patent Application Serial Nos. 60/213,946, filed on Jun. 23, 2000, and 60/241,245, filed on Oct. 17, 2000, the disclosure of each of which is incorporated herein by reference in its entirety for all purposes.

----- KWIC -----

Detail Description Paragraph - DETX (11):

[0111] A nucleic acid, protein, peptide, polypeptide, or other component is "isolated" when it is partially or completely separated from components with which it is normally associated (other peptides, polypeptides, proteins (including complexes, e.g., polymerases and ribosomes which may accompany a native sequence), nucleic acids, cells, synthetic reagents, cellular contaminants, cellular components, etc.), e.g., such as from other components with which it is normally associated in the cell from which it was originally derived. A nucleic acid, polypeptide, or other component is isolated when it is partially or completely recovered or separated from other components of its natural environment such that it is the predominant species present in a composition, mixture, or collection of components (i.e., on a molar basis it is more abundant than any other individual species in the composition). In preferred embodiments, the preparation consists of more than about 70% or 75%, typically more than about 80%, or preferably more than about 90% of the isolated species.

Detail Description Paragraph - DETX (123):

[0221] The polynucleotide sequences of the present invention can be engineered in order to alter an NCSM coding sequence of the invention for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation and/or pegylation patterns, to change codon preference, to introduce splice sites, etc. Further details regarding silent and conservative substitutions are provided below.

Detail Description Paragraph - DETX (129):

[0227] The polynucleotides of the present invention and fragments and variants thereof, which encode the NCSM polypeptide molecules, may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40, bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adeno-associated virus, retroviruses and many others. Any vector that transduces genetic material into a cell, and, if replication is desired, which is replicable and viable in the relevant host can be used.



Detail Description Paragraph - DETX (267):

[0360] Oligonucleotide directed mutagenesis can be used to introduce site-specific mutations in a nucleic acid sequence of interest. Examples of such techniques are found in the references above and, e.g., in Reidhaar-Olson et al. (1988) Science, 241:53-57. Similarly, cassette mutagenesis can be used in a process that replaces a small region of a double stranded DNA molecule with a synthetic oligonucleotide cassette that differs from the native sequence. The oligonucleotide can contain, e.g., completely and/or partially randomized native sequence(s).

Detail Description Paragraph - DETX (396):

[0487] In order to produce antisera for use in an immunoassay, one or more of the immunogenic polypeptides is produced and purified as described herein. For example, recombinant protein may be produced in a mammalian cell line. An inbred strain of mice (used in this assay because results are more reproducible due to the virtual genetic identity of the mice) is immunized with the immunogenic protein(s) in combination with a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, supra, for a standard description of antibody generation, immunoassay formats and conditions that can be used to determine specific immunoreactivity). Alternatively, one or more synthetic or recombinant polypeptides derived from the sequences disclosed herein is conjugated to a carrier protein and used as an immunogen.

Detail Description Paragraph - DETX (695):

[0768] The CD28BP gene is inserted into the polylinker of a pMax Vax vector as described above, forming the first expression unit. The nucleic acid sequence of the cancer antigen, here the polynucleotide encoding the extracellular domain of EpCAM/KSA (or mutant or variant thereof), is linked to a second mammalian expression promoter (exemplary promoters include those set forth in this Example above and elsewhere) and a second poly A signal (exemplary signals include those set forth in this Example above and elsewhere) to form the second expression unit. In this Example, a synthetic poly A (SPA) sequence was made and used. However, one of skill in the art would understand that other poly A sequences (e.g., bovine growth hormone (BGH) poly A or SV40 poly A sequence) can also be used. The synthetic poly A was derived from a sequence for the rabbit .beta.-globin poly A (Gen&Dev. 3:1019-1025 (1989)). The sequence fragment was generated by annealing two oligonucleotides, which contained the respective cloning sites in the 5' and 3' sequences. The upper strand sequence is:  
5'-GATCTGTTTAAACTCTGGCTAATAAAGATCAGAGCTCTAGACATCTGTGTGT  
GGTTTTTTGTGTGTCTCACTACAGA-3', and the sequence of the lower oligonucleotide strand is: 5'-TGAGTGAGACACAAAAACCAACACACAGATGTCTAGAGCT- CTGATCT  
TTTATTAGCCAGAGTTTAAACA-3".

Detail Description Paragraph - DETX (696):

[0769] The second expression unit can be cloned into 3 different sites in the construct pMax Vax-CD28BP, both in forward or reverse orientation: (i)

downstream of the first expression unit (e.g., CMV promoter-CD28BP-SPA polyA, CMVpromoter-CD28BP-BGH polyA, or CMVpromoter-CD28BP-SV40 polyA) using the single cloning sites DraIII and Ascl in pMax Vax10.1; (ii) between the ColE1 and Kana.sup.r gene using the single restriction sites NgoMI and NheI; (iii) between the Kana.sup.r gene and the CMV promoter into the single EcoRV and BsrGI restriction sites (see vector description above in this Example). Independent of the location of the second expression unit it is advisable to add a terminator sequence downstream of the first expression unit. A consensus terminator sequence 5'-ATCAAAA/TTAGGAAGA3' is described in Ming-Chei Maa et al. (1990) JBC 256 (21):12513-12519. In the construct pMax Vax,CD28BP the sequence can be placed into the single DraIII site downstream of the poly A sequence (e.g., synthetic poly A or SPABGH poly A sequence) (see FIG. 22B).

PGPUB-DOCUMENT-NUMBER: 20030138438

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138438 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mericle, Barbara	Edmonds	WA	US	
Fanger, Gary R.	Mill Creek	WA	US	
Vedvick, Thomas S.	Federal Way	WA	US	
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Watanabe, Yoshihiro	Mercer Island	WA	US	
Henderson, Robert A.	Edmonds	WA	US	
Kalos, Michael D.	Seattle	WA	US	
Spies, A. Gregory	Shoreline	WA	US	
Foy, Teresa M.	Federal Way	WA	US	
Fan, Liqun	Bellevue	WA	US	
Wang, Tongtong	Medina	WA	US	

APPL-NO: 10/ 117982

DATE FILED: April 5, 2002

RELATED-US-APPL-DATA:

child 10117982 A1 20020405

parent continuation-in-part-of 10007700 20011130 US PENDING

child 10007700 20011130 US

parent continuation-in-part-of 09897778 20010628 US PENDING

child 09897778 20010628 US

parent continuation-in-part-of 09850716 20010507 US PENDING

child 09850716 20010507 US

parent continuation-in-part-of 09735705 20001212 US PENDING

child 09735705 20001212 US

parent continuation-in-part-of 09685696 20001009 US PENDING

child 09685696 20001009 US  
parent continuation-in-part-of 09662786 20000915 US ABANDONED  
child 09662786 20000915 US  
parent continuation-in-part-of 09643597 20000821 US GRANTED  
parent-patent 6426072 US  
child 09643597 20000821 US  
parent continuation-in-part-of 09630940 20000802 US PENDING  
child 09630940 20000802 US  
parent continuation-in-part-of 09606421 20000628 US PENDING  
child 09606421 20000628 US  
parent continuation-in-part-of 09542615 20000404 US PENDING  
child 09542615 20000404 US  
parent continuation-in-part-of 09510376 20000222 US PENDING  
child 09510376 20000222 US  
parent continuation-in-part-of 09480884 20000110 US PENDING  
child 09480884 20000110 US  
parent continuation-in-part-of 09476496 19991230 US PENDING  
child 09476496 19991230 US  
parent continuation-in-part-of 09466396 19991217 US PENDING  
child 09466396 19991217 US  
parent continuation-in-part-of 09285479 19990402 US PENDING  
child 09285479 19990402 US  
parent continuation-in-part-of 09221107 19981222 US PENDING  
child 09221107 19981222 US  
parent continuation-in-part-of 09123912 19980727 US GRANTED  
parent-patent 6312695 US  
child 09123912 19980727 US

parent continuation-in-part-of 09040802 19980318 US ABANDONED

US-CL-CURRENT: 424/185.1, 424/277.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

----- KWIC -----

Summary of Invention Paragraph - BSTX (567):

[0562] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030129207

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030129207 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of colon cancer

PUBLICATION-DATE: July 10, 2003

US-CL-CURRENT: 424/277.1, 424/185.1, 435/183, 435/320.1, 435/325  
, 435/69.3, 536/23.2

APPL-NO: 10/ 225486

DATE FILED: August 20, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60343517 20011221 US

non-provisional-of-provisional 60314221 20010821 US

PGPUB-DOCUMENT-NUMBER: 20030129192

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030129192 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of ovarian cancer

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chenault, Ruth A.	Seattle	WA	US	
Xu, Jiangchun	Bellevue	WA	US	
Fanger, Gary R.	Federal Way	WA	US	
Harlocker, Susan L.		US		
McNeill, Patricia D.		US		

APPL-NO: 10/ 212677

DATE FILED: August 2, 2002

RELATED-US-APPL-DATA:

child 10212677 A1 20020802

parent continuation-in-part-of 09970966 20011002 US PENDING

child 09970966 20011002 US

parent continuation-in-part-of 09825294 20010403 US PENDING

child 09825294 20010403 US

parent continuation-in-part-of 09713550 20001114 US PENDING

child 09713550 20001114 US

parent continuation-in-part-of 09656668 20000907 US PENDING

child 09656668 20000907 US

parent continuation-in-part-of 09640173 20000815 US PENDING

child 09640173 20000815 US

parent continuation-in-part-of 09561778 20000501 US ABANDONED

child 09561778 20000501 US

parent continuation-in-part-of 09394374 19990910 US ABANDONED

US-CL-CURRENT: 424/155.1, 435/183, 435/320.1, 435/325, 435/6, 435/69.1  
, 435/7.23, 530/350, 536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

----- KWIC -----

Summary of Invention Paragraph - BSTX (242):

[0238] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.



PGPUB-DOCUMENT-NUMBER: 20030125536

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030125536 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of breast cancer

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fanger, Gary R.	Mill Creek	WA	US	
Hirst, Shannon Kathleen	Kirkland	WA	US	
Dillon, Davin C.	Issaquah	WA	US	
Foy, Teresa M.	Federal Way	WA	US	
Houghton, Raymond L.	Bothell	WA	US	
Persing, David H.	Redmond	WA	US	
Kalos, Michael D.	Seattle	WA	US	

APPL-NO: 10/ 212679

DATE FILED: August 2, 2002

RELATED-US-APPL-DATA:

child 10212679 A1 20020802

parent continuation-in-part-of 10079137 20020220 US PENDING

child 10079137 20020220 US

parent continuation-in-part-of 09924400 20010807 US PENDING

child 09924400 20010807 US

parent continuation-in-part-of 09810936 20010316 US PENDING

child 09810936 20010316 US

parent continuation-in-part-of 09699295 20001026 US PENDING

child 09699295 20001026 US

parent continuation-in-part-of 09590583 20000608 US PENDING

child 09590583 20000608 US

parent continuation-in-part-of 09577505 20000524 US PENDING

child 09577505 20000524 US

parent continuation-in-part-of 09534825 20000323 US PENDING

child 09534825 20000323 US

parent continuation-in-part-of 09429755 19991028 US PENDING

child 09429755 19991028 US

parent continuation-in-part-of 09289198 19990409 US PENDING

child 09289198 19990409 US

parent continuation-in-part-of 09062451 19980417 US GRANTED

parent-patent 6344550 US

child 09062451 19980417 US

parent continuation-in-part-of 08991789 19971211 US GRANTED

parent-patent 6225054 US

child 08991789 19971211 US

parent continuation-in-part-of 08838762 19970409 US ABANDONED

child 08838762 19970409 US

parent continuation-in-part-of 08700014 19960820 US ABANDONED

child 08700014 19960820 US

parent continuation-in-part-of 08585392 19960111 US ABANDONED

US-CL-CURRENT: 536/23.2, 435/183 , 435/320.1 , 435/325 , 435/6 , 435/69.1  
 , 435/7.23 , 530/350

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

----- KWIC -----

Detail Description Paragraph - DETX (113):

[0176] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030124140

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030124140 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of ovarian cancer

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bangur, Chaitanya S.	Seattle	WA	US	
Retter, Marc W.	Carnation	WA	US	
Fanger, Gary R.	Mill Creek	WA	US	
Hill, Paul	Duvall	WA	US	

APPL-NO: 10/ 198053

DATE FILED: July 17, 2002

RELATED-US-APPL-DATA:

child 10198053 A1 20020717

parent continuation-in-part-of 09907969 20010717 US PENDING

child 09907969 20010717 US

parent continuation-in-part-of 09884441 20010618 US PENDING

child 09884441 20010618 US

parent continuation-in-part-of 09827271 20010404 US PENDING

child 09827271 20010404 US

parent continuation-in-part-of 09667857 20000920 US PENDING

child 09667857 20000920 US

parent continuation-in-part-of 09636801 20000810 US ABANDONED

child 09636801 20000810 US

parent continuation-in-part-of 09617747 20000717 US PENDING

child 09617747 20000717 US

parent continuation-in-part-of 09404879 19990924 US GRANTED

parent-patent 6468546 US

child 09404879 19990924 US

parent continuation-in-part-of 09338933 19990623 US GRANTED

parent-patent 6488931 US

child 09338933 19990623 US

parent continuation-in-part-of 09216003 19981217 US PENDING

child 09338933 19990623 US

parent continuation-in-part-of 09215681 19981217 US PENDING

US-CL-CURRENT: 424/185.1, 435/183, 435/320.1, 435/325, 435/6, 435/69.3  
, 435/7.23, 536/23.2

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

----- KWIC -----

#### Detail Description Paragraph - DETX (306):

[0369] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030121070

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030121070 A1

TITLE: Genes for modifying plant traits IV

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Adam, Luc	Hayward	CA	US	
Keddie, James	San Mateo	CA	US	
Creelman, Robert	Castro Valley	CA	US	
Riechmann, Jose Luis	Oakland	CA	US	
Jiang, Cai-Zhong	Freemont	CA	US	
Heard, Jacqueline	San Mateo	CA	US	
Pilgrim, Marsha L.	Freemont	CA	US	
Dubell, Arnold	San Leandro	CA	US	
Ratcliffe, Oliver	Oakland	CA	US	
Reuber, T. Lynne	San Mateo	CA	US	
Yu, Guo-Liang	Berkeley	CA	US	
Pineda, Omaira	Vero Beach	FL	US	

APPL-NO: 09/ 934455

DATE FILED: August 22, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60227439 20000822 US

US-CL-CURRENT: 800/278, 536/23.6

ABSTRACT:

The invention relates to plant transcription factor polypeptides, polynucleotides that encode them, homologs from a variety of plant species, and methods of using the polynucleotides and polypeptides to produce transgenic plants having advantageous properties compared to a reference plant. Sequence information related to these polynucleotides and polypeptides can also be used in bioinformatic search methods and is also disclosed.

[0001] This application claims priority benefit of: prior U.S. application entitled "Plant Trait Modification III," serial No. 60/227,439, filed Aug. 22, 2000; prior U.S. application entitled "Genes for Modifying Plant Traits," attorney docket number MBI-0022, serial No. \_\_\_\_\_, filed Nov. 16, 2000; and prior U.S. application entitled "Genes for Modifying Plant Traits II," serial No. 09/837,944, filed Apr. 18, 2001. The entire content of each of these applications is hereby incorporated by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX (73):

[0070] The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Summary of Invention Paragraph - BSTX (74):

[0071] Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

Summary of Invention Paragraph - BSTX (119):

[0116] Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Pat. No. 4,987,071 and U.S. Pat. No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

PGPUB-DOCUMENT-NUMBER: 20030118599

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030118599 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Algate, Paul A.	Issaquah	WA	US	
Lodes, Michael J.	Seattle	WA	US	
Wang, Tongtong	Medina	WA	US	
Fan, Liqun	Bellevue	WA	US	
McNeill, Patricia D.	Federal Way	WA	US	

APPL-NO: 10/ 144649

DATE FILED: May 10, 2002

RELATED-US-APPL-DATA:

child 10144649 A1 20020510

parent continuation-in-part-of 09854133 20010511 US PENDING

child 09854133 20010511 US

parent continuation-in-part-of 09738973 20001214 US PENDING

child 09738973 20001214 US

parent continuation-in-part-of 09704512 20001101 US PENDING

child 09704512 20001101 US

parent continuation-in-part-of 09667170 20000920 US PENDING

child 09667170 20000920 US

parent continuation-in-part-of 09640878 20000818 US ABANDONED

child 09640878 20000818 US

parent continuation-in-part-of 09588937 20000605 US ABANDONED

child 09588937 20000605 US



parent continuation-in-part-of 09538037 20000329 US ABANDONED

child 09538037 20000329 US

parent continuation-in-part-of 09518809 20000303 US ABANDONED

child 09518809 20000303 US

parent continuation-in-part-of 09476235 19991230 US ABANDONED

child 09476235 19991230 US

parent continuation-in-part-of 09370838 19990809 US GRANTED

parent-patent 6444425 US

child 09370838 19990809 US

parent continuation-in-part-of 09285323 19990402 US ABANDONED

US-CL-CURRENT: 424/185.1, 435/183, 435/320.1, 435/325, 435/6, 435/69.1  
, 435/7.23, 536/23.2

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

#### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. patent application Ser. No. 09/854,133, filed May 11, 2001; U.S. patent application Ser. No. 09/738,973, filed Dec. 14, 2000; U.S. patent application Ser. No. 09/704,512, filed Nov. 1, 2000; U.S. patent application Ser. No. 09/667,170, filed Sep. 20, 2000; U.S. Provisional Application No. 60/229,763, filed Sep. 1, 2000; U.S. patent application Ser. No. 09/640,878, filed Aug. 18, 2000; U.S. patent application Ser. No. 09/588,937, filed Jun. 5, 2000; U.S. patent application Ser. No. 09/538,037, filed Mar. 29, 2000; U.S. patent application Ser. No. 09/518,809, filed Mar. 3, 2000; U.S. patent application Ser. No. 09/476,235, filed Dec. 30, 1999; U.S. patent application Ser. No. 09/370,838, filed Aug. 9, 1999; and U.S. patent application Ser. No. 09/285,323, filed Apr. 2, 1999, each a CIP of the previous application and all pending, and incorporated herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX (823):

[0820] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030109434

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030109434 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of kidney cancer

PUBLICATION-DATE: June 12, 2003

US-CL-CURRENT: 514/12, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 530/350  
, 536/23.1

APPL-NO: 10/ 102524

DATE FILED: March 19, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60343340 20011221 US

non-provisional-of-provisional 60277245 20010319 US

PGPUB-DOCUMENT-NUMBER: 20030104366

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030104366 A1

TITLE: Compositions for the treatment and diagnosis of breast cancer and methods for their use

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Jiang, Yuqiu	Kent	WA	US	
Dillon, Davin C.	Redmond	WA	US	
Mitcham, Jennifer L.	Redmond	WA	US	
Xu, Jiangchun	Bellevue	WA	US	
Harlocker, Susan L.	Seattle	WA	US	

APPL-NO: 09/ 551621

DATE FILED: April 17, 2000

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09551621 A1 20000417

parent continuation-in-part-of 09433826 19991103 US PENDING

child 09433826 19991103 US

parent continuation-in-part-of 09389681 19990902 US PENDING

child 09389681 19990902 US

parent continuation-in-part-of 09339338 19990623 US PENDING

child 09339338 19990623 US

parent continuation-in-part-of 09285480 19990402 US PENDING

child 09285480 19990402 US

parent continuation-in-part-of 09222575 19981228 US GRANTED

parent-patent 6387697 US

US-CL-CURRENT: 435/6, 514/44

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as breast cancer, are disclosed. Compositions may comprise one or more breast tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a breast tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as breast cancer. Diagnostic methods based on detecting a breast tumor protein, or mRNA encoding such a protein, in a sample are also provided.

#### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/433,836, filed on Nov. 3, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/389,681, filed on Sep. 2, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/389,338, filed on Jun. 23, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/285,480, filed on Apr. 2, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/222,575, filed Dec. 28, 1998.

----- KWIC -----

#### Detail Description Paragraph - DETX (504):

[0528] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

#### Detail Description Paragraph - DETX (588):

[0612] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20030103994

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030103994 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

PUBLICATION-DATE: June 5, 2003

US-CL-CURRENT: 424/185.1

APPL-NO: 10/ 114666

DATE FILED: April 1, 2002

RELATED-US-APPL-DATA:

child 10114666 A1 20020401

parent continuation-in-part-of 09895828 20010628 US PENDING

PGPUB-DOCUMENT-NUMBER: 20030100748

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030100748 A1

TITLE: Plant promoter derived from luminal binding protein  
gene and methods for its use

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Misra, Santosh	Victoria	CA		
Forward, Benjamin S.	Bocabec	CA		

APPL-NO: 10/ 235113

DATE FILED: September 4, 2002

RELATED-US-APPL-DATA:

child 10235113 A1 20020904

parent continuation-in-part-of 10117641 20020403 US PENDING

child 10117641 20020403 US

parent division-of 09632538 20000804 US GRANTED

parent-patent 6440674 US

US-CL-CURRENT: 536/23.6, 435/320.1 , 435/419 , 435/69.1 , 530/370 , 800/288

ABSTRACT:

Disclosed is a luminal binding protein promoter (PmBiPPro1) including deletions, fusions, and variants thereof. The promoter can be used to direct expression of transgenes.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This is a continuation-in-part of Application No. 10/117,641 filed Apr. 3, 2002, which is a division of application Ser. No. 09/632,538 filed Aug. 4, 2000, now Patent No. 6,440,674, all herein incorporated by reference in their entirety.

----- KWIC -----

Detail Description Paragraph - DETX (102):

[0126] One of ordinary skill in the art will appreciate that a DNA sequence, such as a PmBiP promoter, can be altered in numerous ways without affecting the biological activity of the promoter. For example, PCR can be used to produce variations in a PmBiP promoter DNA sequence. Such variants can be variants optimized for codon preference in a host cell used to express the protein, or other sequence changes that facilitate expression.

Detail Description Paragraph - DETX (186):

[0196] The DNA constructs of the disclosure, containing the PmBiP promoter (SEQ ID NO: 31) or fragments thereof (such as SEQ ID NOS: 16, 17 and 18) operably linked to one or more transgenes may be either homologous or heterologous to the host in question. If homologous to the host cell, i.e., if the transgene is produced by the host cell in nature, then the construct may be connected operably to a different secretory signal sequence and/or terminator sequence than in the natural environment. In this context, the term "homologous" is intended to include a cDNA sequence encoding a transgene that is native to the host cell. The term "heterologous" is intended to include a transgene not expressed by the host cell in nature. Thus, the DNA sequence may be from another organism, or it may be a synthetic sequence.



PGPUB-DOCUMENT-NUMBER: 20030100106

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030100106 A1

TITLE: Baculovirus produced Plasmodium falciparum vaccine

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chang, Sandra P.	Honolulu	HI	US	
Hashimoto, Ann	Kaneohe	HI	US	
Nishimura, Tani	Honolulu	HI	US	

APPL-NO: 10/ 062809

DATE FILED: February 1, 2002

RELATED-US-APPL-DATA:

child 10062809 A1 20020201

parent continuation-in-part-of 09500376 20000208 US PENDING

non-provisional-of-provisional 60266281 20010201 US

US-CL-CURRENT: 435/350, 424/191.1 , 435/183

ABSTRACT:

Compositions and methods are provided for the induction of a protective immunize response in primates against a lethal challenge of Plasmodium.

[0001] This application is a continuation-in-part of application Ser. No.: 09/500,376, filed Feb. 8, 2000, now pending and claims priority to provisional Application No.: 60/266,281, filed Feb. 1, 2001.

----- KWIC -----

Summary of Invention Paragraph - BSTX (9):

[0008] Although a number of investigators have designed and tested gp195-based synthetic peptides and recombinant products as vaccine antigens, no strongly protective vaccine has resulted. Thus, synthetic peptides corresponding to various segments of the N-terminal 83 kDa processing fragment of gp195 induced antibodies in rabbits which displayed only a low level of cross reactivity with asexual blood stage parasites (Cheung et al 1986. Proc. Natl. Acad. Sci. USA 83:8328). One of these synthetic peptides,

corresponding to a non-repetitive, conserved sequence, partially protected Saimiri monkeys against Plasmodium falciparum challenge (Cheung et al. 1986). In a vaccination study in Aotus monkeys using an 83 kDa processing fragment-based recombinant polypeptide produced in E. coli there was no significant difference between the course of infection of control animals and animals immunized with the recombinant polypeptide. In addition, very low levels of antibodies cross-reactive with native gp195 by immunofluorescence were induced (Knapp et al. 1988. Behring Inst. Mitt. 82:349). A bacterial recombinant polypeptide based on a fusion of two conserved regions located towards the amino terminus and center of the gp195 molecule induced only low indirect fluorescent antibody (IFA) titers when used to immunize Aotus monkeys (Herrera et al. 1990. Proc. Natl. Acad. Sci. USA 87:4017) and two out of five immunized animals were partially protected.

#### Summary of Invention Paragraph - BSTX (12):

[0011] A mixture of three synthetic peptides, one peptide from the 83 kDa processing fragment of gp195 and two non-gp195 malaria peptides, partially to completely protected monkeys against parasite challenge; a hybrid synthetic polymer including the sequences of the three synthetic peptides in addition to a circumsporozoite region was reported to provide a delay or suppression of parasitemias (Patarroyo et al. 1987. Nature 328:629; Rodriguez et al. 1990. Am. J. Trop. Med. Hyg. 43:339; Patarroyo et al. 1988. Nature 332:158). Field trials of this hybrid are under way. It is unclear whether any gp195 epitopes in the mixture or hybrid resulted in any protection. In addition there have been two reported studies which were unable to duplicate the prior results obtained using the peptide mixture (Reubush et al. 1990. Am. J. Trop. Med. Hyg. 43:355-366) or the hybrid peptide multimer (Herrera et al. 1991. Abstract in the IV International Congress on Malaria and Babesiosis). Thus, there has been no gp195-based recombinant or synthetic vaccine antigen which has been shown sufficiently effective against Plasmodium falciparum challenge.

#### Detail Description Paragraph - DETX (159):

[0192] The K1 type (p42-K) of the p42 antigen was constructed using the Vietnam-Oak Knoll P. falciparum isolate (FVO). There are three parts to this construct: a leader sequence, the p42-K coding region and the histidine tag (FIG. 13). Restriction sites were incorporated into the primers to enable a "sticky-end" ligation of the three fragments. The leader sequence was altered from the original p42-M sequence such that three adenines were added three bases prior to the start site to optimize the codon preference for baculovirus and insect cells as well as the distance between the promoter sequence and the methionine start codon (Ranjan et al. 1995. Virus Genes 9(2):149-153). Primers containing NarI and PstI restriction site sequences were used to amplify the 1,065 base pair p42-K coding region corresponding to the Ala.sub.1349 to Ser.sub.1723 (as numbered by Miller et al. 1993. Mol. Biochem. Parasitol 59(1):1-14.) of MSP-1 from genomic P. falciparum DNA. Primers containing BamHI and NarI restriction site sequences were used to amplify the 91 base pair leader sequence. Oligonucleotides containing PstI and KpnI restriction site sequences were made to generate the 25 base pair histidine tag. All primers and oligonucleotide sequences used for the p42-K constructs are shown in Table 1.

PGPUB-DOCUMENT-NUMBER: 20030096390

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030096390 A1

TITLE: Novel lipase genes

PUBLICATION-DATE: May 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Giver, Lorraine J.	Santa Clara	CA	US	
Minshull, Jeremy	Menlo Park	CA	US	
Vogel, Kurt	Palo Alto	CA	US	

APPL-NO: 09/ 905666

DATE FILED: July 13, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60217954 20000713 US

non-provisional-of-provisional 60300378 20010621 US

US-CL-CURRENT: 435/198, 435/135 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

New lipase enzymes (both nucleic acids and polypeptides) are provided. Compositions which include these polypeptides, proteins, nucleic acids, recombinant cells, as well as methods involving the enzymes, antibodies to the enzymes, and methods of using the enzymes are also provided.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] Pursuant to 35 USC .sctn.119(e), this application claims priority to and benefit of U.S. Provisional Patent Application Serial Nos. 60/217,954, filed on Jul. 13, 2000, and No. 60/300,378, filed on Jun. 21, 2001, the disclosures of each of which is incorporated herein in their entirety for all purposes.

----- KWIC -----

Detail Description Paragraph - DETX (10):

[0048] A nucleic acid, protein, peptide, polypeptide, or other component is "isolated" when it is partially or completely separated from components with which it is normally associated (such as, other peptides, polypeptides, proteins (including complexes, e.g., polymerases and ribosomes which may

accompany a native sequence), nucleic acids, cells, synthetic reagents, cellular contaminants, cellular components, etc.), e.g., such as from other components with which it is normally associated in the cell from which it was originally derived. A nucleic acid, polypeptide, or other component is substantially pure when it is partially or completely recovered or separated from other components of its natural environment such that it is the predominant species present in a composition, mixture, or collection of components (i.e., on a molar basis it is more abundant than any other individual species in the composition). In preferred embodiments, the preparation consists of more than 70%, typically more than 80%, or preferably more than 90% of the isolated species.

Detail Description Paragraph - DETX (68):

[0106] The polynucleotide sequences of the present invention can be engineered in order to alter lipase homologue coding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns or other conjugation patterns, to change codon preference, to introduce splice sites, to introduce or remove introns, etc.

Detail Description Paragraph - DETX (74):

[0112] The polynucleotides of the present invention may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adeno-associated virus, retroviruses and many others. Any vector that transduces genetic material into a cell, and, if replication is desired, which is replicable and viable in the relevant host can be used.

Detail Description Paragraph - DETX (194):

[0229] Oligonucleotide directed mutagenesis can be used to introduce site-specific mutations in a nucleic acid sequence of interest. Examples of such techniques are found in the references above and, e.g., in Reidhaar-Olson et al. (1988) Science, 241:53-57. Similarly, cassette mutagenesis can be used in a process that replaces a small region of a double stranded DNA molecule with a synthetic oligonucleotide cassette that differs from the native sequence. The oligonucleotide can contain, e.g., completely and/or partially randomized native sequence(s).

Detail Description Paragraph - DETX (257):

[0292] In order to produce antisera for use in an immunoassay, one or more of the immunogenic polypeptides is produced and purified as described herein. For example, recombinant protein may be produced in a mammalian cell line. An inbred strain of mice (used in this assay because results are more reproducible due to the virtual genetic identity of the mice) is immunized with the

immunogenic protein(s) in combination with a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see, Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a standard description of antibody generation, immunoassay formats and conditions that can be used to determine specific immunoreactivity). Alternatively, one or more **synthetic or recombinant polypeptide derived from the sequences** disclosed herein is conjugated to a carrier protein and used as an immunogen.

PGPUB-DOCUMENT-NUMBER: 20030087818

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087818 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of colon cancer

PUBLICATION-DATE: May 8, 2003

US-CL-CURRENT: 514/12, 435/183, 435/320.1, 435/325, 435/69.1, 536/23.2

APPL-NO: 10/ 066543

DATE FILED: February 1, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60313077 20010816 US

non-provisional-of-provisional 60290322 20010511 US

non-provisional-of-provisional 60267400 20010202 US

non-provisional-of-provisional 60305265 20010712 US

non-provisional-of-provisional 60267382 20010207 US

PGPUB-DOCUMENT-NUMBER: 20030087317

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087317 A1

TITLE: Human NIM1 kinase

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bandman, Olga	Mountain View	CA	US	
Molteni, Angela	Cantu	MI	IT	
Magnaghi, Paola	Nerviano		US	
Bosotti, Roberta	Ann Arbor		IT	
Scacheri, Emanuela			US	
Isacchi, Antonella			US	
Hodgson, David M.			US	

APPL-NO: 10/ 195101

DATE FILED: July 11, 2002

RELATED-US-APPL-DATA:

child 10195101 A1 20020711

parent continuation-in-part-of 09523849 20000313 US GRANTED

parent-patent 6458561 US

US-CL-CURRENT: 435/7.23, 435/194 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention provides a human NIM1 kinase, an encoding nucleic acid molecule, and an antibody that specifically binds the protein. The invention also provides for the use of these compositions in the characterization, diagnosis, and treatment of disorders associated NIM1 kinase expression.

[0001] This application claims priority to U.S. Ser. No. 09/523,849, filed Mar. 13, 2000.

----- KWIC -----

Detail Description Paragraph - DETX (27):

[0062] "Protein" refers to an amino acid sequence, oligopeptide, peptide, polypeptide or portions thereof whether naturally occurring or synthetic.

Detail Description Paragraph - DETX (61):

[0096] A multitude of nucleic acid molecules encoding NIM1 kinase may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The nucleic acid sequence can be engineered by such methods as DNA shuffling (U.S. Pat. No. 5,830,721) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change **codon preference** to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (**promoters**, enhancers, specific initiation signals, and polyadenylated 3' sequence) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid molecule, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).



PGPUB-DOCUMENT-NUMBER: 20030083480

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030083480 A1

TITLE: Novel glyphosate N-acetyl transferase (GAT) genes

PUBLICATION-DATE: May 1, 2003

US-CL-CURRENT: 536/23.1

APPL-NO: 10/ 004357

DATE FILED: October 29, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60244385 20001030 US

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Provisional Patent Application Serial No. 60/244,385 filed Oct. 30, 2000, the disclosure of which is incorporated herein by reference in its entirety for all purposes.

PGPUB-DOCUMENT-NUMBER: 20030078396

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030078396 A1

TITLE: Compositions and methods for the detection, diagnosis  
and therapy of hematological malignancies

PUBLICATION-DATE: April 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gaiger, Alexander	Seattle	WA	US	
Algate, Paul A.	Issaquah	WA	US	
Mannion, Jane	Edmonds	WA	US	
Retter, Marc	Carnation	WA	US	

APPL-NO: 10/ 040862

DATE FILED: November 6, 2001

RELATED-US-APPL-DATA:

child 10040862 A1 20011106

parent continuation-in-part-of 09796692 20010301 US PENDING

non-provisional-of-provisional 60223378 20000807 US

non-provisional-of-provisional 60223416 20000804 US

non-provisional-of-provisional 60222903 20000803 US

non-provisional-of-provisional 60218950 20000714 US

non-provisional-of-provisional 60206201 20000522 US

non-provisional-of-provisional 60202084 20000504 US

non-provisional-of-provisional 60202084 20000504 US

non-provisional-of-provisional 60200999 20000501 US

non-provisional-of-provisional 60200303 20000428 US

non-provisional-of-provisional 60200779 20000428 US

non-provisional-of-provisional 60200545 20000427 US

non-provisional-of-provisional 60190479 20000317 US

non-provisional-of-provisional 60186126 20000301 US

US-CL-CURRENT: 536/23.1

#### ABSTRACT:

Disclosed are methods and compositions for the detection, diagnosis, prognosis, and therapy of hematological malignancies, and in particular, B cell leukemias, lymphomas and multiple myelomas. Disclosed are compositions, methods and kits for eliciting immune and T cell responses to specific malignancy-related antigenic polypeptides and antigenic polypeptide fragments thereof in an animal. Also disclosed are compositions and methods for use in the identification of cells and biological samples containing one or more hematological malignancy-related compositions, and methods for the detection and diagnosis of such diseases and affected cell types. Also disclosed are diagnostic and therapeutic kits, as well as methods for the diagnosis, therapy and/or prevention of a variety of leukemias and lymphomas.

#### CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation in part of U.S. Ser. No. 09/796,692 filed Mar. 1, 2001, which claims priority to United States Provisional Patent Application Serial Nos. 60/186,126, filed Mar. 1, 2000; Serial No. 60/190,479, filed Mar. 17, 2000; Serial No. 60/200,545, filed Apr. 27, 2000; Serial No. 60/200,303, filed Apr. 28, 2000; Serial No. 60/200,779, filed Apr. 28, 2000; Serial No. 60/200,999, filed May 1, 2000; Serial No. 60/202,084, filed May 4, 2000; Serial No. 60/206,201, filed May 22, 2000; Serial No. 60/218,950, filed Jul. 14, 2000; Serial No. 60/222,903, filed Aug. 3, 2000; Serial No. 60/223,416, filed Aug. 4, 2000; and Serial No. 60/223,378, filed Aug. 7, 2000; the entire specification, claims, sequences and figures of each of which is specifically incorporated herein by reference in its entirety without disclaimer and for all purposes.

----- KWIC -----

#### Detail Description Paragraph - DETX (181):

[0228] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

#### Detail Description Paragraph - DETX (256):

[0294] The end result of the flow of genetic information is the synthesis of

protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20030073144

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030073144 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of pancreatic cancer

PUBLICATION-DATE: April 17, 2003

US-CL-CURRENT: 435/7.23, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

APPL-NO: 10/ 060036

DATE FILED: January 30, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60333626 20011127 US

non-provisional-of-provisional 60305484 20010712 US

non-provisional-of-provisional 60265305 20010130 US

non-provisional-of-provisional 60267568 20010209 US

non-provisional-of-provisional 60313999 20010820 US

non-provisional-of-provisional 60291631 20010516 US

non-provisional-of-provisional 60287112 20010428 US

non-provisional-of-provisional 60278651 20010321 US

non-provisional-of-provisional 60265682 20010131 US

PGPUB-DOCUMENT-NUMBER: 20030069180

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030069180 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of colon cancer

PUBLICATION-DATE: April 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Jiang, Yuqiu	Kent	WA	US	
Harlocker, Susan L.	Seattle	WA	US	
Secrist, Heather	Seattle	WA	US	
Wang, Aijun	Issaquah	WA	US	
Stolk, John A.	Bothell	WA	US	

APPL-NO: 10/ 146502

DATE FILED: May 14, 2002

RELATED-US-APPL-DATA:

child 10146502 A1 20020514

parent continuation-in-part-of 10046935 20020115 US PENDING

child 10046935 20020115 US

parent continuation-in-part-of 09878178 20010608 US PENDING

non-provisional-of-provisional 60270216 20010220 US

non-provisional-of-provisional 60210899 20000609 US

US-CL-CURRENT: 514/12, 435/320.1 , 435/325 , 435/69.3 , 435/7.23 , 530/350  
, 536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

----- KWIC -----

Summary of Invention Paragraph - BSTX (167):

[0162] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030068327

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030068327 A1

TITLE: Compositions and methods for the diagnosis and  
threatment of herpes simplex virus infection

PUBLICATION-DATE: April 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hosken, Nancy Ann	Seattle	WA	US	
McGowan, Patrick	Seattle	WA	US	
Sleath, Paul R.	Seattle	WA	US	
Mossman, Sally P.	Seattle	WA	US	
Evans, Lawrence S.	Seattle	WA	US	
Swanson, Ryan M.	Seattle	WA	US	
McNeill, Patricia D.	Federal Way	WA	US	

APPL-NO: 10/ 121988

DATE FILED: April 11, 2002

RELATED-US-APPL-DATA:

child 10121988 A1 20020411

parent continuation-in-part-of 09894998 20010628 US PENDING

non-provisional-of-provisional 60277438 20010320 US

non-provisional-of-provisional 60215458 20000629 US

US-CL-CURRENT: 424/186.1, 435/219, 435/69.1, 536/23.72

ABSTRACT:

Compounds and methods for the diagnosis and treatment of HSV infection are provided. The compounds comprise polypeptides that contain at least one antigenic portion of an HSV polypeptide and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits are also provided comprising such polypeptides and/or DNA sequences and a suitable detection reagent for the detection of HSV infection in patients and in biological samples.

----- KWIC -----



Summary of Invention Paragraph - BSTX (245):

[0241] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX (329):

[0325] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

Detail Description Paragraph - DETX (240):

[0693] CD8.sup.+ T cells isolated from a panel of HSV-2 seropositive donors were screened for their ability to respond to a variety of HSV-2 proteins. Briefly, PBMCs were obtained from donors EB5491, AG10295, LM10295, and 447, and enriched for CD8.sup.+ T cells using microbeads or CD8+ Enrichment Kits from Miltenyi. Synthetic peptides (15 amino acids in length and overlapping in sequence by 10 or 11 amino acids) were synthesized across several complete or partial ORFs from HSV-2 strain HG52, including proteins UL21 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 144 and 154, respectively), UL50 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 145 and 153, respectively), US3 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 146 and 154, respectively), UL54 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 147 and 156, respectively), US8 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 148 and 157, respectively), UL19 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 149 and 158, respectively), UL46 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 150 and 159, respectively), UL18 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 151 and 160, respectively), and RL2 (the full length DNA/amino acids of which are disclosed in SEQ ID NOs: 152 and 161, respectively). The peptides were screened by 24 co-culture of the donor's CD8+T cells (2-5.times.10.sup.5 cells/well), autologous dendritic cells (2-5.times.10.sup.4 cells/well) and peptides (0.5 .mu.g/ml each) in 96-well ELISPOT plates that had been pre-coated with anti-human IFN- $\gamma$  antibody. Each peptide pool was screened in an individual well. The ELISPOT plates were developed as per a standard

protocol. The number of spots per well was counted using an automated video-microscopy ELISPOT reader. Individual 15-mer peptides, determined from peptide pools testing positive, were screened as described above and returned the following results:

PGPUB-DOCUMENT-NUMBER: 20030064947

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030064947 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

PUBLICATION-DATE: April 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wang, Tongtong	Medina	WA	US	
Wang, Aijun	Issaquah	WA	US	
Skeiky, Yasir A. W.	Bellevue	WA	US	
Li, Samuel X.	Redmond	WA	US	
Kalos, Michael D.	Seattle	WA	US	
Henderson, Robert A.	Edmonds	WA	US	
McNeill, Patricia D.	Federal Way	WA	US	
Fanger, Neil	Seattle	WA	US	
Retter, Marc W.	Carnation	WA	US	
Durham, Margarita	Seattle	WA	US	
Fanger, Gary R.	Mill Creek	WA	US	
Vedvick, Thomas S.	Federal Way	WA	US	
Carter, Darrick	Seattle	WA	US	
Watanabe, Yoshihiro	Mercer Island	WA	US	
Peckham, David W.	Seattle	WA	US	
Cai, Feng	Lake Forest Park	WA	US	
Foy, Teresa M.	Federal Way	WA	US	

APPL-NO: 10/ 007700

DATE FILED: November 30, 2001

RELATED-US-APPL-DATA:

child 10007700 A1 20011130

parent continuation-in-part-of 09897778 20010628 US PENDING

child 09897778 20010628 US

parent continuation-in-part-of 09850716 20010507 US PENDING

child 09850716 20010507 US

parent continuation-in-part-of 09735705 20001212 US PENDING

child 09735705 20001212 US

parent continuation-in-part-of 09685696 20001009 US PENDING  
child 09685696 20001009 US

parent continuation-in-part-of 09662786 20000915 US PENDING  
child 09662786 20000915 US

parent continuation-in-part-of 09643597 20000821 US PENDING  
child 09643597 20000821 US

parent continuation-in-part-of 09630940 20000802 US PENDING  
child 09630940 20000802 US

parent continuation-in-part-of 09606421 20000628 US PENDING  
child 09606421 20000628 US

parent continuation-in-part-of 09542615 20000404 US PENDING  
child 09542615 20000404 US

parent continuation-in-part-of 09510376 20000222 US PENDING  
child 09510376 20000222 US

parent continuation-in-part-of 09480884 20000110 US PENDING  
child 09480884 20000110 US

parent continuation-in-part-of 09476496 19991230 US PENDING  
child 09476496 19991230 US

parent continuation-in-part-of 09466396 19991217 US PENDING  
child 09466396 19991217 US

parent continuation-in-part-of 09285479 19990402 US PENDING  
child 09285479 19990402 US

parent continuation-in-part-of 09221107 19981222 US PENDING  
child 09221107 19981222 US

parent continuation-in-part-of 09123912 19980727 US GRANTED

parent-patent 6312695 US

child 09123912 19980727 US

parent continuation-in-part-of 09040802 19980318 US PENDING

US-CL-CURRENT: 514/44, 424/93.21

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/897,778 filed Jun. 28, 2001; which is a continuation-in-part of U.S. patent application Ser. No. 09/850,716 filed May 7, 2001; which is a continuation-in-part of U.S. patent application Ser. No. 09/735,705 filed Dec. 12, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/685,696 filed Oct. 9, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/662,786 filed Sep. 15, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/643,597 filed Aug. 21, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/630,940 filed Aug. 2, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/606,421 filed Jun. 28, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/542,615 filed Apr. 4, 2000; which is a continuation-in-part of U.S. patent application No. 09/510,376 filed Feb. 22, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/480,884 filed Jan. 10, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/476,496 filed Dec. 30, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/466,396 filed Dec. 17, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/285,479 filed Apr. 2, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/221,107 filed Dec. 22, 1998; which is a continuation-in-part of U.S. patent application Ser. No. 09/123,912 filed Jul. 27, 1998; which is a continuation-in-part of U.S. patent application Ser. No. 09/040,802 filed Mar. 18, 1998 and all incorporated by reference herein.

----- KWIC -----

Detail Description Paragraph - DETX (111):

[0548] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to

engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030059781

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030059781 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of ovarian and endometrial cancer

PUBLICATION-DATE: March 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chenault, Ruth A.	Seattle	WA	US	
Xu, Jiangchun	Bellevue	WA	US	

APPL-NO: 09/ 997279

DATE FILED: November 28, 2001

RELATED-US-APPL-DATA:

child 09997279 A1 20011128

parent continuation-in-part-of 09813358 20010320 US PENDING

non-provisional-of-provisional 60257276 20001219 US

non-provisional-of-provisional 60213748 20000622 US

non-provisional-of-provisional 60190710 20000321 US

US-CL-CURRENT: 435/6, 536/24.3

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as ovarian or endometrial cancer, are disclosed. Compositions may comprise one or more ovarian carcinoma proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses such an antigen, or a T cell that is specific for cells expressing such an antigen. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian and endometrial cancer. Diagnostic methods based on detecting an ovarian carcinoma protein, or mRNA encoding such an antigen, in a sample are also provided.

----- KWIC -----

Summary of Invention Paragraph - BSTX (260):

[0256] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX (344):

[0340] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.



PGPUB-DOCUMENT-NUMBER: 20030056245

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030056245 A1

TITLE: AP1 amine oxidase variants

PUBLICATION-DATE: March 20, 2003

US-CL-CURRENT: 800/279, 435/228 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2

APPL-NO: 10/ 072307

DATE FILED: February 6, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60266918 20010206 US

non-provisional-of-provisional 60300324 20010622 US

#### CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] Pursuant to 35 USC .sctn.119(e), this application claims priority to and benefit of U.S. Provisional Patent Application Serial Nos. 60/266,918, filed on Feb. 6, 2001, and 60/300,324, filed on Jun. 22, 2001, the disclosures of each of which are incorporated herein in their entirety for all purposes.

PGPUB-DOCUMENT-NUMBER: 20030054363

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054363 A1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Henderson, Robert A.	Edmonds	WA	US	
Wang, Tongtong	Medina	WA	US	
Watanabe, Yoshihiro	Mercer Island	WA	US	
Johnson, Jeffrey C.	Des Moines	WA	US	
Retter, Marc W.	Carnation	WA	US	
Durham, Margarita	Seattle	WA	US	
Carter, Darrick	Seattle	WA	US	
Fanger, Gary R.	Mill Creek	WA	US	
Vedvick, Thomas S.	Federal Way	WA	US	
Bangur, Chaitanya S.	Seattle	WA	US	
McNabb, Andria	Renton	WA	US	

APPL-NO: 10/ 017754

DATE FILED: October 29, 2001

RELATED-US-APPL-DATA:

child 10017754 A1 20011029

parent continuation-in-part-of 09902941 20010710 US PENDING

child 09902941 20010710 US

parent continuation-in-part-of 09849626 20010503 US PENDING

child 09849626 20010503 US

parent continuation-in-part-of 09736457 20001213 US PENDING

child 09736457 20001213 US

parent continuation-in-part-of 09702705 20001030 US PENDING

child 09702705 20001030 US

parent continuation-in-part-of 09677419 20001006 US PENDING

child 09677419 20001006 US

parent continuation-in-part-of 09671325 20000926 US PENDING

child 09671325 20000926 US

parent continuation-in-part-of 09658824 20000908 US PENDING

child 09658824 20000908 US

parent continuation-in-part-of 09651563 20000829 US PENDING

child 09651563 20000829 US

parent continuation-in-part-of 09614124 20000711 US PENDING

child 09614124 20000711 US

parent continuation-in-part-of 09589184 20000605 US PENDING

child 09589184 20000605 US

parent continuation-in-part-of 09560406 20000427 US PENDING

child 09560406 20000427 US

parent continuation-in-part-of 09546259 20000410 US PENDING

child 09546259 20000410 US

parent continuation-in-part-of 09533077 20000322 US PENDING

child 09533077 20000322 US

parent continuation-in-part-of 09519642 20000306 US PENDING

child 09519642 20000306 US

parent continuation-in-part-of 09476300 19991230 US PENDING

child 09476300 19991230 US

parent continuation-in-part-of 09466867 19991217 US PENDING

child 09466867 19991217 US

parent continuation-in-part-of 09419356 19991015 US PENDING

child 09419356 19991015 US

parent continuation-in-part-of 09346492 19990630 US PENDING

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 435/7.23

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/902,941, filed Jul. 10, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/849,626, filed May 3, 2001; which is a continuation-in-part of U.S. patent application Ser. No. 09/736,457, filed Dec. 13, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/702,705, filed Oct. 30, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/677,419, filed Oct. 6, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/671,325, filed Sept. 26, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/658,824, filed Sept. 8, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/651,563, filed Aug. 29, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/614,124, filed Jul. 11, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/589,184, filed Jun. 5, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/560,406, filed Apr. 27, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/546,259, filed Apr. 10, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/533,077, filed Mar. 22, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/519,642, filed Mar. 6, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/476,300, filed Dec. 30, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/466,867, filed Dec. 17, 1999; which is a continuation-in-part of U.S. patent application 09/419,356, filed Oct. 15, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/346,492, filed Jun. 30, 1999, all now pending, which applications are incorporated herein by reference in their entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX (1167):

[1164] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may

be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX (92):

[1388] To facilitate the generation, purification, and evaluation of MAb against L985P, MAbs against the entire deduced amino acid sequence of the L985P protein, peptides derived from L985P or chemically produced (synthetic) L985P peptides will be used. Also, one can use MAbs raised against chimeric forms of L985P protein molecule fused to Ra12 protein, either the long form (Ra12--which is the first 128 amino acids of Ra12) and/or the short form (Ra12S), or fused to a polyhistidine peptide or any combination of these molecules. Provided are the predicted cDNA and amino acid sequences for the his-tagged L985P-Ra12 fusion molecules: Ra12-L985P\_cDNA (SEQ ID NO:1875), Ra12-L985P\_Protein (SEQ ID NO:1876), Ra12S-L985P\_cDNA (SEQ ID NO:1877) and Ra12S-L985P\_Protein (SEQ ID NO:1878); and the L985P derived peptides: his-tagged Ra12S-L985PEX\_cDNA (SEQ ID NO:1879), his-tagged Ra12S-L985PEX\_Protein (SEQ ID NO:1880), L985P\_Extracellular\_Loop-2\_cDNA (SEQ ID NO:1 88 1) and L985P\_Extracellular\_Loop-2\_Peptide (SEQ ID NO:1 882).

PGPUB-DOCUMENT-NUMBER: 20030049827

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030049827 A1

TITLE: Subtilisin variants

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ness, Jon E.	Sunnyvale	CA	US	
Welch, Mark	Fremont	CA	US	
Giver, Lorraine J.	Santa Clara	CA	US	
Cherry, Joel R.	Davis	CA	US	
Borchert, Torben V.	Birkeroed	CA	DK	
Stemmer, Willem P.C.	Los Gatos	CA	US	
Minshull, Jeremy	Menlo Park		US	

APPL-NO: 09/ 824893

DATE FILED: April 2, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60194143 20000403 US

US-CL-CURRENT: 435/226, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

New subtilisin homologues (both nucleic acids and proteins) are provided. Compositions which include these new proteins, recombinant cells, shuffling methods involving the new homologues, antibodies to the new homologues, and methods of using the homologues are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to and benefit of U.S. Provisional Application 60/194,143, filed Apr. 3, 2000, the disclosure of which is incorporated herein in its entirety for all purposes.

----- KWIC -----

Detail Description Paragraph - DETX (35):

[0062] The polynucleotide sequences of the present invention can be engineered in order to alter a subtilisin homologue coding sequence for a variety of reasons, including but not limited to, alterations which modify the

cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques that are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, alter glycosylation patterns, change codon preference, introduce splice sites, etc.

Detail Description Paragraph - DETX (41):

[0068] Polynucleotides of the present invention can be incorporated into any one of a variety of expression vectors suitable for expressing a polypeptide. Suitable vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adenovirus, adeno-associated virus, retroviruses and many others. Any vector that transduces genetic material into a cell, and, if replication is desired, which is replicable and viable in the relevant host can be used.

Detail Description Paragraph - DETX (145):

[0172] Oligonucleotide directed mutagenesis can be used to introduce site-specific mutations in a nucleic acid sequence of interest. Examples of such techniques are found in the references above and, e.g., in Reidhaar-Olson et al. (1988) Science, 241:53-57. Similarly, cassette mutagenesis can be used in a process that replaces a small region of a double stranded DNA molecule with a synthetic oligonucleotide cassette that differs from the native sequence. The oligonucleotide can contain, e.g., completely and/or partially randomized native sequence(s).

Detail Description Paragraph - DETX (191):

[0218] In order to produce antisera for use in an immunoassay, one or more of the immunogenic polypeptides is produced and purified as described herein. For example, recombinant protein may be produced in a bacterial cell line. An inbred strain of mice (used in this assay because results are more reproducible due to the virtual genetic identity of the mice) is immunized with the immunogenic protein(s) in combination with a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see, Harlow and Lane (1988) Antibodies A Laboratory Manual, Cold Spring Harbor Publications, New York, for a standard description of antibody generation, immunoassay formats and conditions that can be used to determine specific immunoreactivity). Alternatively, one or more synthetic or recombinant polypeptide derived from the sequences disclosed herein is conjugated to a carrier protein and used as an immunogen.

PGPUB-DOCUMENT-NUMBER: 20030041356

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030041356 A1

TITLE: Methods for modifying flowering phenotypes

PUBLICATION-DATE: February 27, 2003

US-CL-CURRENT: 800/290, 536/23.6

APPL-NO: 09/ 819142

DATE FILED: March 27, 2001



PGPUB-DOCUMENT-NUMBER: 20030028920

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030028920 A1

TITLE: Antimicrobial polypeptides and their uses

PUBLICATION-DATE: February 6, 2003

US-CL-CURRENT: 800/279, 435/320.1 , 435/419 , 435/6 , 435/69.1 , 530/350  
, 536/23.2

APPL-NO: 10/ 125258

DATE FILED: April 18, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60285355 20010420 US

#### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/285,355 filed Apr. 20, 2001, which application is hereby incorporated herein in its entirety by reference.

US-PAT-NO: 6646109

DOCUMENT-IDENTIFIER: US 6646109 B1

TITLE: Cloning and expression of a novel acetylcholine-gated  
ion channel receptor subunit

DATE-ISSUED: November 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Elgoyhen; Ana Belen	Del Mar	CA	N/A	N/A
Johnson; David S.	La Jolla	CA	N/A	N/A
Boulter; James Richard	San Diego	CA	N/A	N/A
Heinemann; Stephen Fox	La Jolla	CA	N/A	N/A

APPL-NO: 09/ 345109

DATE FILED: June 29, 1999

PARENT-CASE:

RELATED INVENTIONS

This application is a divisional application of U.S. application Ser. No. 08/464,258, filed Jun. 5, 1995, now U.S. Pat. No. 6,013,766, issued Jan. 11, 2000 which is a divisional application of U.S. application Ser. No. 08/278,635, filed Jul. 21, 1994, now U.S. Pat. No. 5,683,912, issued Nov. 4, 1997. This application cross-references U.S. application Ser. No. 07/898,185, filed Jun. 12, 1992, now U.S. Pat. No. 5,371,188, issued Dec. 6, 1994, and U.S. application Ser. No. 07/664,473, filed Mar. 4, 1991, now abandoned, which is a continuation of U.S. application Ser. No. 07/321,384, filed Mar. 14, 1989, now abandoned, which is a continuation of U.S. application Ser. No. 07/170,295, filed Mar. 18, 1988, now abandoned, each of which are incorporated by reference herein in their entirety.

US-CL-CURRENT: 530/388.22, 530/389.1

ABSTRACT:

The present invention provides isolated nucleic acids encoding alpha9 nicotinic acetylcholine receptor subunit and receptor subunit protein encoded thereby. Also provided are vectors containing the invention nucleic acids, host cells transformed therewith, alpha9 nicotinic acetylcholine receptor subunit and functional nicotinic acetylcholine receptors comprising at least one alpha9 subunit expressed recombinantly in such host cells as well as transgenic non-human mammals that express the invention receptor subunit and mutants thereof. Receptors of the invention comprise at least one alpha9 nicotinic acetylcholine subunit and form cationic channels activated by acetylcholine, but blocked by nicotine and muscarine. The invention also

provides methods for identifying compounds that modulate the ion channel activity of the functional invention receptors containing at least one invention subunit.

14 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

----- KWIC -----

Drawing Description Text - DRTX (53):

Vectors employed in the present invention contain both a **promoter** and a cloning site into which nucleic acid encoding alpha9 receptor subunit(s) can be operatively linked. Such vectors, which are well known in the art, are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, J. Biol. Chem. 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for native codons of the alpha9 nAChR subunit in order to enhance transcription (e.g., the **codon preference** of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Drawing Description Text - DRTX (54):

Examples of suitable vectors that may be employed in the present invention include viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Invention nucleic acids are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic linkers can be ligated to the termini of restricted invention nucleic acids. These **synthetic linkers contain nucleic acid sequences** that correspond to a particular restriction site in the vector DNA. Additionally, a nucleic acid containing a termination codon and an appropriate restriction site can be ligated into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA.

Other means are well known and available in the art.

US-PAT-NO: 6632636

DOCUMENT-IDENTIFIER: US 6632636 B1

TITLE: Nucleic acids encoding 3-ketoacyl-ACP reductase from  
Moraxella catarrhalis

DATE-ISSUED: October 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lagace; Robert E.	Belmont	CA	N/A	N/A
Patterson; Chandra	Menlo Park	CA	N/A	N/A
Berg; Kim L.	Palo Alto	CA	N/A	N/A

APPL-NO: 09/ 596002

DATE FILED: June 16, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority under 35 U.S.C..sctn.119(e) to U. S.  
Provisional Application Serial No. 60/140,121, filed Jun. 18, 1999.

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US-CL-CURRENT: 435/69.1, 435/189, 435/252.3, 435/254.11, 435/320.1  
, 435/325, 435/440, 435/70.1, 435/71.1, 536/23.2  
, 536/24.32

ABSTRACT:

The present invention provides the genomic sequences of a library of  
purified nucleic acid molecules, or their complements, comprising the genome of  
Moraxella catarrhalis. The invention also provides the identification of open  
reading frames contained within the nucleic acid molecules of the library. The  
present invention further provides for the use of the nucleic acid molecules,  
their complements or fragments, and proteins or portions thereof for  
identifying ligands and useful diagnostic and therapeutic compositions. In  
addition the invention provides for vectors, host cells and methods for  
producing M. catarrhalis proteins or portions thereof.

14 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (38):

"Protein" refers to an amino acid sequence, oligopeptide, peptide, polypeptide or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX (17):

A nucleic acid molecule encoding a *M. catarrhalis* protein may be cloned into a vector and used to express the protein or portions thereof in host cells. The nucleic acid sequence can be engineered by such methods as DNA shuffling (U.S. Pat. No. 5,830,721) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and polyadenylated sequence) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid molecule, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6630574

DOCUMENT-IDENTIFIER: US 6630574 B1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

DATE-ISSUED: October 7, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wang; Tongtong	Medina	WA	N/A	N/A
Bangur; Chaitanya S.	Seattle	WA	N/A	N/A
Lodes; Michael J.	Seattle	WA	N/A	N/A
Fanger; Gary R.	Mill Creek	WA	N/A	N/A
Vedvick; Thomas S.	Federal Way	WA	N/A	N/A
Carter; Darrick	Seattle	WA	N/A	N/A
Retter; Marc W.	Carnation	WA	N/A	N/A
Mannion; Jane	Edmonds	WA	N/A	N/A
Fan; Liquan	Bellevue	WA	N/A	N/A

APPL-NO: 09/ 614124

DATE FILED: July 11, 2000

PARENT-CASE:

REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 09/589,184, filed Jun. 5, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/560,406, filed Apr. 27, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/546,259, filed Apr. 10, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/533,077, filed Mar. 22, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/519,642 filed Mar. 6, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/476,300, filed Dec. 30, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/466,867, filed Dec. 17, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/419,356, filed Oct. 15, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/346,492, filed Jun. 30, 1999.

US-CL-CURRENT: 530/350, 424/184.1 , 530/300

ABSTRACT:

Compositions comprising isolated cancer-associated L552S polypeptides are disclosed. Such compositions can comprise, for example, portions of said cancer-associated L552S polypeptides and/or the corresponding polynucleotides

encoding such portions. Illustrative uses of the compositions, for example in cancer diagnostic methods, are also provided.

2 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (889):

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Brief Summary Text - BSTX (973):

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.



US-PAT-NO: 6627739

DOCUMENT-IDENTIFIER: US 6627739 B1

TITLE: .beta.-secretase enzyme compositions and methods

DATE-ISSUED: September 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Anderson; John P.	San Francisco	CA	N/A	N/A
Basi; Guriqbal	Palo Alto	CA	N/A	N/A
Doan; Minh Tam	Hayward	CA	N/A	N/A
Frigon; Normand	Milbrae	CA	N/A	N/A
John; Varghese	San Francisco	CA	N/A	N/A
Power; Michael	Fremont	CA	N/A	N/A
Sinha; Sukanto	San Francisco	CA	N/A	N/A
Tatsuno; Gwen	Oakland	CA	N/A	N/A
Tung; Jay	Belmont	CA	N/A	N/A
Wang; Shuwen	Hersey	PA	N/A	N/A
McConlogue; Lisa	Burlingame	CA	N/A	N/A

APPL-NO: 09/ 724566

DATE FILED: November 28, 2000

PARENT-CASE:

This application is a continuation of U.S. application Ser. No. 09/501,708 filed Feb. 10, 2000, which claims the benefit of U.S. Provisional Application Nos. 60/119,571 filed Feb. 10, 1999, now abandoned, and 60/139,172 filed Jun. 15, 1999, now abandoned, all of which are hereby incorporated herein by reference in their entireties.

US-CL-CURRENT: 530/387.9, 530/388.1, 530/388.26, 530/389.1, 530/389.2

ABSTRACT:

Disclosed are various forms of an active, isolated .beta.-secretase enzyme in purified and recombinant form. This enzyme is implicated in the production of amyloid plaque components which accumulate in the brains of individuals afflicted with Alzheimer's disease. Recombinant cells that produce this enzyme either alone or in combination with some of its natural substrates (.beta.-APPwt and .beta.-APPsw) are also disclosed, as are antibodies directed to such proteins. These compositions are useful for use in methods of selecting compounds that modulate .beta.-secretase. Inhibitors of .beta.-secretase are implicated as therapeutics in the treatment of neurodegenerative diseases, such as Alzheimer's disease.

1 Claims, 57 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 48

----- KWIC -----

Detailed Description Text - DETX (203):

The polynucleotide sequences of the present invention can be engineered in order to alter a .beta.-secretase coding sequence for a variety of reasons, including but not limited to, alterations that modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to produce splice variants, etc. For example, it may be advantageous to produce .beta.-secretase -encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray, E. et al. (1989) Nuc Acids Res 17:477-508) can be selected, for example, to increase the rate of .beta.-secretase polypeptide expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence. This may be particularly useful in producing recombinant enzyme in non-mammalian cells, such as bacterial, yeast, or insect cells. The present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are also described in Sambrook, et al., (supra).

Detailed Description Text - DETX (206):

The polynucleotides of the present invention may be included in any of a variety of expression vectors suitable for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculoviruses; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host. The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and related sub-cloning procedures are deemed to be within the scope of those skilled in the art.

US-PAT-NO: 6620922

DOCUMENT-IDENTIFIER: US 6620922 B1

TITLE: Compositions and methods for the therapy and diagnosis  
of prostate cancer

DATE-ISSUED: September 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Xu; Jiangchun	Bellevue	WA	N/A	N/A
Dillon; Davin C.	Issaquah	WA	N/A	N/A
Mitcham; Jennifer L.	Redmond	WA	N/A	N/A
Harlocker; Susan L.	Seattle	WA	N/A	N/A
Jiang; Yuqiu	Kent	WA	N/A	N/A
Kalos; Michael D.	Seattle	WA	N/A	N/A
Fanger; Gary R.	Mill Creek	WA	N/A	N/A
Retter; Marc W.	Carnation	WA	N/A	N/A
Stolk; John A.	Bothell	WA	N/A	N/A
Day; Craig H.	Seattle	WA	N/A	N/A
Vedvick; Thomas S.	Federal Way	WA	N/A	N/A
Carter; Darrick	Seattle	WA	N/A	N/A
Li; Samuel X.	Redmond	WA	N/A	N/A
Wang; Aijun	Issaquah	WA	N/A	N/A
Skeiky; Yasir A. W.	Bellevue	WA	N/A	N/A
Hepler; William T.	Seattle	WA	N/A	N/A
Henderson; Robert A.	Edmonds	WA	N/A	N/A

APPL-NO: 09/ 636215

DATE FILED: August 9, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. application Ser. No. 09/605,783, filed Jun. 27, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/593,793, filed Jun. 13, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/570,737, filed May 12, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/568,100, filed May 9, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/536,857, filed Mar. 27, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/483,672, filed Jan. 14, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/443,686, filed Nov. 18, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/439,313, filed Nov. 12, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/352,616, filed Jul. 13, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/288,946, filed Apr. 9, 1999, which is a continuation-in-part of

U.S. patent application No. 09/232,149, filed Jan. 15, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/159,812, filed Sep. 23, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 09/115,453, filed Jul. 14, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 09/030,607, filed Feb. 25, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 09/020,956, filed Feb. 9, 1998.

US-CL-CURRENT: 536/23.1, 435/6, 435/91.1, 536/23.4, 536/23.5, 536/24.1, 536/24.31, 536/24.33

#### ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as prostate cancer, are disclosed. Compositions may comprise one or more prostate-specific proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a prostate-specific protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as prostate cancer. Diagnostic methods based on detecting a prostate-specific protein, or mRNA encoding such a protein, in a sample are also provided.

10 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

----- KWIC -----

#### Detailed Description Text - DETX (39):

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

#### Detailed Description Text - DETX (124):

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA

segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

US-PAT-NO: 6617109

DOCUMENT-IDENTIFIER: US 6617109 B1

TITLE: Compositions and methods for the therapy and diagnosis  
of ovarian cancer

DATE-ISSUED: September 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Xu; Jiangchun	Bellevue	WA	N/A	N/A
Stolk; John A.	Bothell	WA	N/A	N/A

APPL-NO: 09/ 713550

DATE FILED: November 14, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 09/656,668, filed Sep. 7, 2000, which is a CIP of U.S. application Ser. No. 09/640,173, filed Aug. 15, 2000, which is a CIP of U.S. application Ser. No. 09/561,778, filed May 1, 2000, now abandoned which is a CIP of U.S. application Ser. No. 09/394,374, filed Sep. 10, 1999, now abandoned all pending and incorporated by reference in their entirety herein.

US-CL-CURRENT: 435/6, 435/91.2, 436/64, 514/23, 514/44, 536/24.3  
, 536/24.33

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 1,3

----- KWIC -----

Brief Summary Text - BSTX (151):

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

US-PAT-NO: 6613515

DOCUMENT-IDENTIFIER: US 6613515 B1

TITLE: Ovarian tumor sequences and methods of use therefor

DATE-ISSUED: September 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Xu; Jiangchun	Bellevue	WA	N/A	N/A
Stolk; John A.	Bothell	WA	N/A	N/A

APPL-NO: 09/ 640173

DATE FILED: August 15, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of the U.S. application Ser. No. 09/561,778, filed May 1, 2000, (now abandoned), which is a continuation-in-part of the U.S. application Ser. No. 09/394,374 (now abandoned), filed Sep. 10, 1999, which are incorporated by reference in their entirety herein.

US-CL-CURRENT: 435/6, 435/91.2, 435/91.21, 436/501, 436/64, 436/813, 436/94, 536/23.1, 536/23.5, 536/24.3, 536/24.31, 536/24.33, 536/25.3

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer, are disclosed. Compositions may comprise one or more ovarian carcinoma proteins, portions thereof, polynucleotides that encode such portions or antibodies or immune system cells specific for such proteins. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian cancer. Polypeptides and polynucleotides as provided herein may further be used for the detection and monitoring of ovarian cancer.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1,2

----- KWIC -----

Brief Summary Text - BSTX (129):



Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

US-PAT-NO: 6586572

DOCUMENT-IDENTIFIER: US 6586572 B2

TITLE: Compositions and methods for the therapy and diagnosis  
of breast cancer

DATE-ISSUED: July 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jiang; Yuqiu	Kent	WA	N/A	N/A
Dillon; Davin C.	Issaquah	WA	N/A	N/A
Mitcham; Jennifer L.	Redmond	WA	N/A	N/A
Xu; Jiangchun	Bellevue	WA	N/A	N/A
Harlocker; Susan L.	Seattle	WA	N/A	N/A
Hepler; William T.	Seattle	WA	N/A	N/A

APPL-NO: 09/ 604287

DATE FILED: June 22, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 09/590,751, filed Jun. 8, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/551,621, filed Apr. 17, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/433,828, filed on Nov. 3, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/389,681, filed on Sep. 2, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/339,338, filed on Jun. 23, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/285,480, filed on Apr. 2, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/222,575, filed Dec. 28, 1998.

US-CL-CURRENT: 530/350, 530/387.3

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as breast cancer, are disclosed. Compositions may comprise one or more breast tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a breast tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as breast cancer. Diagnostic methods based on detecting a breast tumor protein, or mRNA encoding such a protein, in a sample are also provided.

6 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX (39):

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detailed Description Text - DETX (123):

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

US-PAT-NO: 6537555

DOCUMENT-IDENTIFIER: US 6537555 B2

TITLE: Compositions and methods for the diagnosis and treatment  
of herpes simplex virus infection

DATE-ISSUED: March 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hosken; Nancy A.	Seattle	WA	N/A	N/A
Day; Craig H.	Seattle	WA	N/A	N/A
Dillon; Davin C.	Issaquah	WA	N/A	N/A
McGowan; Patrick	Seattle	WA	N/A	N/A
Sleath; Paul R.	Seattle	WA	N/A	N/A

APPL-NO: 09/ 894998

DATE FILED: June 28, 2001

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is related to U.S. Provisional Application No. 60/277,438 filed Mar. 20, 2001 and U.S. Provisional Application No. 60/215,458 filed Jun. 29, 2000 and are incorporated in their entirety by reference herein.

US-CL-CURRENT: 424/199.1, 424/184.1, 424/186.1, 424/204.1, 424/231.1  
, 435/235.1, 435/320.1, 435/325, 435/6, 536/23.72

ABSTRACT:

Compounds and methods for the diagnosis and treatment of HSV infection are provided. The compounds comprise polypeptides that contain at least one antigenic portion of an HSV polypeptide and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits are also provided comprising such polypeptides and/or DNA sequences and a suitable detection reagent for the detection of HSV infection in patients and in biological samples.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (60):

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Brief Summary Text - BSTX (144):

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

US-PAT-NO: 6531315

DOCUMENT-IDENTIFIER: US 6531315 B1

TITLE: Compositions and methods for the therapy and diagnosis  
of lung cancer

DATE-ISSUED: March 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wang; Tongtong	Medina	WA	N/A	N/A
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Bangur; Chaitanya S.	Seattle	WA	N/A	N/A
Hosken; Nancy A.	Seattle	WA	N/A	N/A
Fanger; Gary R.	Mill Creek	WA	N/A	N/A
Li; Samuel X.	Redmond	WA	N/A	N/A
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Skeiky; Yasir A. W.	Bellevue	WA	N/A	N/A

APPL-NO: 09/ 606421

DATE FILED: June 28, 2000

PARENT-CASE:

REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 09/542,615 filed Apr. 4, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/510,376, filed Feb. 22, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/480,884, filed Jan. 10, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/476,496, filed Dec. 30, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/466,396, filed Dec. 17, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/285,479, filed Apr. 2, 1999, which claims priority from and is a continuation of PCT Application No. PCT/US99/05798, filed Mar. 17, 1999, which claims priority from and is a continuation-in-part of U.S. patent application Ser. No. 09/221,107, filed Dec. 22, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 09/123,912, filed Jul. 27, 1998, now U.S. Pat. No. 6,312,695, which is a continuation-in-part of U.S. patent application Ser. No. 09/040,802, filed Mar. 18, 1998, abandoned May 23, 2001.

US-CL-CURRENT: 435/372.3, 424/184.1, 424/185.1, 435/326

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as

lung cancer, are disclosed. Compositions may comprise one or more lung tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a lung tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as lung cancer. Diagnostic methods based on detecting a lung tumor protein, or mRNA encoding such a protein, in a sample are also provided.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (72):

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Brief Summary Text - BSTX (156):

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

US-PAT-NO: 6528054

DOCUMENT-IDENTIFIER: US 6528054 B1

TITLE: Compositions and methods for the therapy and diagnosis  
of breast cancer

DATE-ISSUED: March 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jiang; Yuqiu	Kent	WA	N/A	N/A
Dillon; Davin C.	Issaquah	WA	N/A	N/A
Mitcham; Jennifer L.	Redmond	WA	N/A	N/A
Xu; Jiangchun	Bellevue	WA	N/A	N/A
Harlocker; Susan L.	Seattle	WA	N/A	N/A
Hepler; William T.	Seattle	WA	N/A	N/A

APPL-NO: 09/ 620405

DATE FILED: July 20, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation in part of U.S. patent application Ser. No. 09/604,287, filed Jun. 22, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/590,751, filed Jun. 8, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/551,621, filed Apr. 17, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/433,826, filed on Nov. 3, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/389,681, filed on Sep. 2, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/339,338, filed on Jun. 23, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/285,480, filed on Apr. 2, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/222,575, filed Dec. 28, 1998.

US-CL-CURRENT: 424/130.1, 530/387.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as breast cancer, are disclosed. Compositions may comprise one or more breast tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a breast tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as breast cancer. Diagnostic methods based on detecting a breast tumor protein, or mRNA



encoding such a protein, in a sample are also provided.

5 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX (518):

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detailed Description Text - DETX (602):

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

US-PAT-NO: 6521413

DOCUMENT-IDENTIFIER: US 6521413 B1

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor

DATE-ISSUED: February 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Daggett; Lorrie P.	San Diego	CA	N/A	N/A
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Liaw; Chen Wang	San Diego	CA	N/A	N/A
Lu; Chin-Chun	San Diego	CA	N/A	N/A

APPL-NO: 09/ 386123

DATE FILED: August 30, 1999

PARENT-CASE:

RELATED APPLICATIONS

This application is a continuation of allowed U.S. application Ser. No. 08/486,273, filed, Jun. 6, 1995, now U.S. Pat. No. 5,985,586 which is a divisional of U.S. Ser. No. 08/231,193, filed Apr. 20, 1994, now U.S. Pat. No. 5,849,895, which is a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now abandoned, the entire contents of each of these applications is herein incorporated by reference.

US-CL-CURRENT: 435/7.2, 435/252.3 , 435/471 , 435/69.1

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises

methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

65 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

----- KWIC -----

Detailed Description Text - DETX (41):

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detailed Description Text - DETX (61):

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which

may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into **synthetic DNA sequences** which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	32075	(humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1)	USPAT; US-PGPUB	2003/12/16 11:41
2	L2	8	1 same (regulat\$8 and codon adj preference)	USPAT; US-PGPUB	2003/12/16 11:42
3	L3	244	(transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon adj preference	USPAT; US-PGPUB	2003/12/16 11:54
4	L4	175	3 and 1	USPAT; US-PGPUB	2003/12/16 11:55
5	L5	353	1 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:08
6	L6	3893	1 same (regulat\$8 or codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:08
7	L7	6442	1 same muta\$10	USPAT; US-PGPUB	2003/12/16 14:08
8	L8	1942	6 and 7	USPAT; US-PGPUB	2003/12/16 14:08
9	L9	52	8 and 5	USPAT; US-PGPUB	2003/12/16 14:09

PGPUB-DOCUMENT-NUMBER: 20030190700

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190700 A1

TITLE: Synthetic nucleic acid molecule for imparting multiple traits

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gonsalves, Dennis	Hilo	HI	US	
Fermin-Munoz, Gustavo Alberto	Hilo	HI	US	

APPL-NO: 10/ 131814

DATE FILED: April 24, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60286075 20010424 US

US-CL-CURRENT: 435/69.1, 435/235.1, 435/320.1, 435/325, 435/6, 530/350, 536/23.2

ABSTRACT:

The present invention is directed to a DNA construct which includes a modified DNA molecule with a nucleotide sequence which is at least 80%, but less than 100%, homologous to two or more desired trait DNA molecules and which imparts the desired trait to plants transformed with the DNA construct. Each of the desired trait DNA molecules relative to the modified nucleic acid molecule have nucleotide sequence similarity values which differ by no more than 3 percentage points. The DNA construct may further include either a silencer or a plurality of modified DNA molecules. The present invention also relates to host cells, plant cells, transgenic plants, and transgenic plant seeds containing such DNA constructs. The present invention is also directed to a method of preparing a modified nucleic acid molecule suitable to impart multiple traits to a plant, a method of determining whether multiple desired traits can be imparted to plants by a single modified DNA molecule, and a method for imparting traits to plants by transforming the plants with the DNA construct.

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/286,075, filed Apr. 24, 2001.

----- KWIC -----

Detail Description Paragraph - DETX (27):

[0069] It is possible for the DNA construct of the present invention to be configured so that the trait and silencer DNA molecules encode RNA molecules which are translatable. As a result, that RNA molecule will be translated at the ribosomes to produce the protein encoded by the DNA construct. Production of proteins in this manner can be increased by joining the cloned gene encoding the DNA construct of interest with synthetic double-stranded oligonucleotides which represent a viral regulatory sequence (i.e., a 5' untranslated sequence) (U.S. Pat. No. 4,820,639 to Gehrke, and U.S. Pat. No. 5,849,527 to Wilson, which are hereby incorporated by reference in their entirety).

Detail Description Paragraph - DETX (47):

[0089] When a modified synthetic nucleotide sequence is selected as having the attributes of a synthetic nucleic acid sequence of the present invention and, therefore, is identified as suitable for use in imparting a trait, the synthetic nucleic acid may be prepared by recombinant methodologies including, without limitation, site mutagenesis of one of the original trait nucleic acids; polymerase chain reaction, using an available original nucleic acid and suitable primers, or other methods of nucleic acid manipulation known in the art. As a preferable alternative, the identified homologized nucleotide sequence of present invention can be synthesized using any DNA synthesizer capable of producing an oligonucleotide of the desired size. Thus, the present invention can be carried out without handling the actual source organism for the trait DNA. This eliminates the need for time-consuming recombinant manipulations, significantly reducing both the effort and cost of producing a suitable modified nucleic acid sequence. The resulting modified nucleotide is subsequently cloned into an expression vector suitable for transformation into a chosen host, as described above.

Detail Description Paragraph - DETX (77):

[0111] All gene fragments were digested with an excess of BamHI and XhoI for no less than 12 hours, according to Sambrook et al., "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor, N.Y.: Cold Spring Harbor Press (1989), which is hereby incorporated by reference in its entirety. The digested fragments were excised from agarose gels and column purified for ligation into the BamHI/XhoI cloning site of vector pEPJ86GFP, shown in FIG. 2. In this cloning vector, a transcriptional fusion with GFP gene was created under the control of a double 35S promoter. For chemical or electro transformation either Escherichia coli DH5.alpha. or XL1-Blue were routinely used. For plasmid miniprep either the alkaline lysis or the boiling method were used. Five independent recombinant plasmids per construct were sequenced; plasmids with identical sequence to the computer-generated gene fragments were chosen for digestion and subsequent subcloning into pGA482G. Digestion with KpnI and HindIII renders a subcloning fragment with the GFP gene fused to the synthetic 3/4 N gene fragment, and under the control of the 35S promoter. After checking by PCR and restriction analysis the recombinant derivatives of pGA482G were sequenced again before transforming Agrobacterium tumefaciens LBA4404. A. tumefaciens transformants were kept at -80.degree. C. after checking for the recombinant plasmid by restriction analysis. PCR products from transgenic plants were also column purified and sequenced from at least three different

transgenic lines. All sequencing was done using an ABI 373 automated sequencer.

Detail Description Paragraph - DETX (95):

[0117] Sequence modifications made to a DNA fragment are generally limited by the availability of restriction sites, but also by the ability of mutating at random, or even site-specifically, the gene under analysis. When trying to establish how important nucleotide changes are, both the number and the location of nucleotide changes must be assessed under controlled conditions. This is, by far, the most important requisite in the design for making synthetic genes with specific desired properties, to test different hypothesis, and to attain specific plant phenotypes. Based on a sequence previously known to confer resistance, new fragments of 216 bp of TSWV N gene were designed with nucleotide changes to render scattered, 5' end, middle and 3' end region constructs differing by 5% and 15%, and scattered 10% and 20% from the native N TSWV-BL gene. A starting assumption was that in modifying the nucleotide sequence of a previous fragment proven to confer resistance in transgenic plants, a point would be reached in which resistance was no longer viable. In this way, information could be gathered regarding what extent of similarity is important to trigger post-transcriptional gene silencing, and, even more importantly, on how the location of nucleotide changes affect the phenotype of transgenic plants when challenged with the homologous virus.

Detail Description Paragraph - DETX (96):

[0118] Using the strategy originally devised by Stemmer et al., "Single-Step Assembly of a Gene and Entire Plasmid from Large Numbers of Oligodeoxyribonucleotides," Gene 164(1):49-53 (1995), which is hereby incorporated by reference in its entirety, the constructs indicated by Table 4 were synthesized. The engineered N gene fragment of TSWV-BL (the lettuce isolate of TSWV) is the nucleotide sequence designated as 3/4N by Pang et al., "Nontarget DNA Sequences Reduce the Transgene Length Necessary for RNA-Mediated Tobacco Virus Resistance in Transgenic Plants," Proc. Natl. Acad. Sci. USA, 94:8261-8266 (1997), which is hereby incorporated by reference in its entirety, which corresponds to bases 2668-2373 (antisense orientation) according to Pang et al., "Resistance to Heterologous Isolates of Tomato Spotted Wilt Virus in Transgenic Plants Expressing its Nucleocapsid Protein Gene," Phytopathology, 82: 1223-29 (1992), which is hereby incorporated by reference in its entirety. All fragments were 216 nt long, but adding the restriction recognition sites and random nucleotides introduced for cloning purposes, all assembled products are 240 nt long. No cloning from native virus was required. Even the native version of the 3/4N gene fragment was synthetic, but also used as controls were 3/4N-transgenic segregating lines previously obtained in this laboratory. In this sense the synthetic N gene fragment here used is identical to the one published by Pang et al., "Resistance to Heterologous Isolates of Tomato Spotted Wilt Virus in Transgenic Plants Expressing its Nucleocapsid Protein Gene," Phytopathology 82: 1223-29 (1992), which is hereby incorporated by reference in its entirety, and all modified constructs derived from this single original sequence. This sequence was chosen as a baseline due to its short size and its ability to confer good level of resistance to TSWV when transcriptionally fused to GFP gene. The design of the oligos and the changes introduced to the N sequence were performed by



computer using the DNASTar software program. For every construct, all oligos required for their synthesis were individually designed and the complementary strand deduced using the DNASTar software. When all oligo sequences were deduced they were introduced in a different program of the same software to allow its virtual assemblage. All of them, without exception, gave a unique config that matches 100% with the intended sequence when assembled as indicated in Table 4. That is, no other assemblage product was produced in this simulation-like exercise. Afterwards, computer-simulated restriction analysis were performed to assess whether the introduced changes created BamHI, XhoI, KpnI, and/or HindIII restriction sites that would eventually complicate the cloning and subcloning process. All 10 newly-created sequences harbored only the restriction sites purposely introduced in the amplification oligos for cloning purposes (BamHI and XhoI). In all cases, the introduced nucleotide changes were transversions; meaning that no overall change in G+C content is expected. The 10 constructs created are listed in Table 4.

Detail Description Paragraph - DETX (105):

[0123] Transgenic plants transformed with 3/4N gene fused to **GFP** can confer resistance to TSWV but not to the related tospovirus Groundnut Ringspot Virus (GRSV). TSWV and GRSV are 78% similar, and it is possible that the lack of resistance of transgenic plants challenged with GRSV is due to a level of homology which is insufficient to target the GRSV genome for degradation, even if post-transcriptional gene silencing is triggered. To further explore the possibility that modifying the TSWV 3/4N transgene would allow broader viral resistance of transgenic plants, a gene was synthesized that is, in toto, approximately 90% similar to the corresponding fragments of both TSWV and GRSV. To design this sequence, a 3/4N TSWV gene was modified to make it more homologous to the GRSV N gene nucleotide sequence. In doing so, of course, the similarity to the native TSWV N gene decreased, the net result being that the artificial sequence presented here is 90% similar to both TSWV N gene and its counterpart in GRSV. The nt 1417-1632 (in sense form) of TSWV and nt 560-775 of GRSV were used. The newly created **synthetic sequence** (called Rec2 herein), when compared with its parental 3/4N TSWV-BL gene sequence, has the following changes: one insertion and one deletion, plus 22 base changes (ca. 10% changes compared with the native sequence of 3/4N TSWV gene). Similarity of TSWV N gene dropped from 100% to 90% according to sequence alignment. Similarity to GRSV rose from 73% to 89% according to the same analysis. The homologous gene of Tomato Chlorotic Spot Virus (TCSV), another member of the same group as TSWV and GRSV, is also 89% similar to Rec2, as seen in Table 8. In FIG. 6, all modifications are highlighted and uninterrupted stretches of full similarity are also indicated.

Detail Description Paragraph - DETX (149):

[0151] A **synthetic sequence** able to target for degradation of all known isolates of PRSV was generated. These isolates come from the Americas, Asia, and the Pacific (i.e., Australia and Hawaii). The desired trait is PRSV resistance in papaya (and probably some cucurbits). Sequences from 47 American isolates, 29 Asian isolates, and 8 from Hawaii and Australia, were used for a total of 84 sequences. These isolates and their accession numbers are shown in Table 18. It has been shown that transgenic papayas are resistant, in most cases, to closely similar strains of the virus (homologous resistance), which

creates the unforeseen disadvantage of a potential low durability of transgenic resistance due to mutation or introduction of new variants of PRSV (Tennant et al., "Papaya Ringspot Virus Resistance of Transgenic Rainbow and Sunup is Affected By Gene Dosage, Plant Development, and Coat Protein Homology," European J. of Plant Pathology 107:645-653 (2001), which is hereby incorporated by reference in its entirety). On the other hand, due to an extreme dependence on sequence similarity, different transgenic lines should, theoretically, be created for every geographical location in which a different variant of the virus is prevalent to keep the virus under control. Such a task, although apparently pragmatic, would be impractical in terms of cost and labor. Furthermore, many isolates of PRSV have been a source of CP genes that have been already cloned, and most of them sequenced.

PGPUB-DOCUMENT-NUMBER: 20030188331

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030188331 A1

TITLE: Regulated gene expression in plants

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

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Ullman, Christopher Graeme	London		NY GB	
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Sanchez, Juan Pablo	New York		US	

APPL-NO: 10/ 192078

DATE FILED: July 10, 2002

RELATED-US-APPL-DATA:

child 10192078 A1 20020710

parent division-of 09732348 20001207 US PENDING

child 09732348 20001207 US

parent continuation-in-part-of PCT/GB00/02071 20000530 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0001578.4	2000GB-0001578.4	January 24, 2000
GB	0001580.0	2000GB-0001580.0	January 24, 2000

US-CL-CURRENT: 800/278, 435/219 , 435/419 , 800/288

ABSTRACT:

A method is provided of regulating transcription in a plant cell from a DNA sequence comprising a target DNA operably linked to a coding sequence, which method comprises introducing an engineered zinc finger polypeptide in said plant cell which polypeptide binds to the target DNA and modulates transcription of the coding sequence.

REFERENCE TO RELATED APPLICATIONS/INCORPORATION BY REFERENCE

[0001] This application is a continuation-in-part of PCT application no. PCT/GB00/02071 entitled "GENE SWITCHES" filed May 30, 2000 designating the US and claiming priority from GB applications 9912635.1 filed May 18, 1999 and

001578.4 filed Jan. 24, 2000. Further mentioned and incorporated by reference herein are PCT/GB99/03730, filed Nov. 9, 1999, published as WO00/27878A1 on May 18, 2000 entitled "Screening System For Zinc Finger Polypeptides For A Desired Binding Ability" and claiming priority from GB application 9824544.2, filed Nov. 9, 1998, and designating the US; PCT/GB99/03730 which is a continuation-in-part of U.S. patent application Ser. No. 09/139,672, filed Aug. 25, 1998 (now U.S. Pat. No. 6,013,453), which is a continuation of U.S. patent application Ser. No. 08/793,408 (now U.S. Pat. No. 6,007,988), filed as PCT application no. PCT/GB95/01949 on Aug. 17, 1995, designating the U.S. and, published as WO96/06166 on Feb. 29, 1996 entitled "Improvements in or Relating to Binding Proteins for Recognition of DNA"; PCT/GB95/01949 claims the benefit of priority from GB application 9514698.1, filed Jul. 19, 1995, GB application 9422534.9, filed Nov. 8, 1994 and GB application no. 9416880.4, filed Aug. 20, 1994. Mention is also made of: U.S. Ser. No. 08/422,107; WO96/32475; WO99/47656A2, published Sep. 23, 1999 entitled "Nucleic Acid Binding Proteins"; WO98/53060A1, published Nov. 26, 1998 entitled "Nucleic Acid Binding Proteins"; WO98/53059A1 published Nov. 26, 1998 entitled "Nucleic Acid Binding Proteins"; WO98/53058A1 published Nov. 26, 1998 entitled "Nucleic Acid Binding Proteins"; WO98/53057A1 published Nov. 26, 1998 ("Nucleic Acid Binding Polypeptide Library"; U.S. Pat. Nos. 6,013,453 and 6,007,988; Fichn et al. (2000) Nature Biotechnol. 18:1157-1161; Richter et al. (2000) Nature Biotechnol. 18:1167-1171; and, generally, Nature Biotechnol. Vol. 18(11) together with all documents cited or referenced therein. Each of the foregoing applications and patents, and each document cited or referenced in each of the foregoing applications and patents, including during the prosecution of each of the foregoing applications and patents ("application cited documents") and any manufacturer's instructions or catalogues for any products cited or mentioned in each of the foregoing applications and patents and in any of the application cited documents, are hereby incorporated herein by reference. Furthermore, all documents cited in this text, and all documents cited or referenced in documents cited in this text, and any manufacturer's instructions or catalogues for any products cited or mentioned in this text, are hereby incorporated herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX (138):

[0167] Any changes that are made to the coding sequence can be made using techniques that are well known in the art and include site directed mutagenesis, PCR, and synthetic gene construction. Such methods are described in published patent applications EP 0 385 962 , EP 0 359 472 and WO 93/07278. Well-known protocols for transient expression in plants can be used to check the expression of modified genes before their transfer to plants by transformation.

Detail Description Paragraph - DETX (196):

[0220] To investigate the utility of heterologous zinc finger proteins for the regulation of plant genes, a synthetic zinc finger protein was designed and introduced into transgenic *A. thaliana* under the control of a promoter capable of expression in a plant as described below. A second construct comprising the

zinc finger protein binding sequence fused upstream of the Green Fluorescent Protein (**GFP**) reporter gene was also introduced into transgenic *A. thaliana* as described in Example 2. Crossing the two transgenic lines produced progeny plants carrying both constructs in which the **GFP** reporter gene was expressed demonstrating transactivation of the gene by the zinc finger protein.

PGPUB-DOCUMENT-NUMBER: 20030182674

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030182674 A1

TITLE: Method of making a genetically modified mammal

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Frazer, Ian Hector	St. Lucia		AU	
Zhou, Jian	Jindalee		AU	
Sun, Xiao Yi	Jindalee		AU	

APPL-NO: 10/ 305633

DATE FILED: November 27, 2002

RELATED-US-APPL-DATA:

child 10305633 A1 20021127

parent division-of 09479645 20000107 US GRANTED

parent-patent 6489141 US

child 10305633 A1 20021127

parent continuation-in-part-of PCT/AU98/00530 19980709 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
AU	PO7765	1997AU-PO7765	July 9, 1997
AU	PO9467	1997AU-PO9467	September 11, 1997
AU	PP8078	1999AU-PP8078	January 8, 1999

US-CL-CURRENT: 800/18, 435/354 , 435/455 , 800/21

ABSTRACT:

A method of making a genetically modified mammal, the method including selecting a first codon of a parent polynucleotide that encodes a polypeptide for replacement with a synonymous codon, wherein the synonymous codon is selected on the basis that it exhibits a higher translational efficiency in a first cell type of the mammal than the first codon in a comparison of translational efficiencies of codons in cells of the first type, replacing the first codon with the synonymous codon to form a synthetic polynucleotide, and introducing the synthetic polynucleotide into a cell of the mammal.

## CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of co-pending U.S. patent application Ser. No. 09/479,645, filed Jan. 7, 2000, in the name of Ian Hector Frazer et al., which claims priority of Australian Patent Application No. PP 8078, filed Jan. 8, 1999, and which is a continuation-in-part of co-pending International Patent Application No. PCT/AU98/00530, filed Jul. 9, 1998, which designates the United States, and which claims priority of Australian Patent Application Nos. P07765, filed Jul. 9, 1997, and P09467, filed Sep. 11, 1997. The subject matter of both related applications PCT/AU98/00530 and 09/479,645 are hereby incorporated herein by reference.

----- KWIC -----

### Brief Description of Drawings Paragraph - DRTX (14):

[0062] FIG. 6A, comprising FIGS. 6A-1, 6A-2, and 6A-3, is a series of images, each of which depicts a confocal micrograph showing expression of GFP in COS-1 cells transfected with wild-type gfp (wt) (FIG. 6A-2) or a synthetic gfp gene (FIG. 6A-3) carrying codons used at relatively high frequency by papillomavirus genes (p). FIG. 6A-1 shows results from a mock transfection.

### Brief Description of Drawings Paragraph - DRTX (16):

[0064] FIG. 7, comprising FIGS. 7A through 7L, is a series of images, each of which depicts the expression pattern of GFP in vivo from wild-type gfp gene, or a synthetic gfp gene carrying codons used at relatively high frequency by papillomavirus genes. Using a gene gun, mice were shot with PGFP (FIGS. 7A through 7F) and GFP (FIGS. 7G through 7L) expression plasmids encoding GFP protein. A transverse section of the mouse skin section shows where the gfp gene is expressed. Bright-field photographs (FIGS. 7A, 7B, 7G, and 7H) of the same section where dermis (D) epidermis (E) are highlighted are shown to identify the location of fluorescence in the epidermis. Arrows indicate fluorescent signals.

### Detail Description Paragraph - DETX (169):

[0230] Results As the major limitation to expression of the wild type BPV L1 and L2 genes appeared to be translational in our system, we wished to test whether this limitation reflected a limited availability of the appropriate tRNA species for gene translation. As transient expression of the synthetic genes within intact cells can be regulated by many factors, we tested our hypothesis in a cell free system using rabbit reticulocyte lysate (RRL) or wheat germ lysate to examine gene translation. Similar amounts of plasmids expressing the wild type or synthetic humanized BPV1 L1 gene were added to a T7-DNA polymerase coupled RRL transcription/translation system in the presence of 35S-methionine. After 20 minutes, translated proteins were separated by SDS PAGE and visualized by autoradiography. Efficient translation of the modified L1 gene was observed (FIG. 4A, lane 2), while translation of the wild type BPV1 L1 sequence resulted in a weak 55 kDa L1 band (FIG. 4A, lane 1). We reasoned that although the wild type sequence was not optimized for translation in RRL,

some translation would occur, as there would be no cellular mRNA species competing for the 'rare' codons present in the wild type L1 sequence. The above data suggest that the observed difference in efficiency of translation of the wild type and synthetic humanized L1 genes is a consequence of limited availability of the tRNAs required for translation of the rare codons present in the wild type gene. We therefore expected that addition of excess tRNA to the in vitro translation system would overcome the inhibition of translation of the wild type L1 gene. To address this question, 10-5 M aminoacyl-tRNAs from yeast were added into the RRL translation system, and L1 protein synthesis was assessed. Introduction of exogenous tRNAs resulted in a dramatic improvement in translation of the wild type L1 sequence, which now gave a yield of L1 protein comparable to that observed with the synthetic humanized L1 sequence (SEQ ID NO: 3) (FIG. 4A). Enhancement of translation of the wild type L1 gene (SEQ ID NO: 1) by aminoacyl-tRNA was dose-dependent, with an optimum efficiency at 10-5 M tRNA. As addition of exogenous tRNA improved the yield of L1 protein translated from the wild type L1 gene sequence (SEQ ID NO: 1), we assessed the speed of translation of wild type and humanized L1 mRNA. Samples were collected from the translation mixture every 2 minutes, starting at the 8th minute. Translation of L1 (SEQ ID NO: 2, 4) from the wild type sequence (SEQ ID NO: 1) was much slower than from the humanized L1 sequence (SEQ ID NO: 3) (FIG. 4B), and the retardation of translation could be completely overcome by adding exogenous tRNA from commercially available yeast tRNA. Yeast tRNA was chosen in the above analysis because the codon usage in yeast is similar to that of papillomavirus (Table 1). Addition of exogenous tRNA did not significantly improve the translation of the humanized L1 gene (SEQ ID NO: 3), indicating that this sequence was optimized with regard to codon usage for the rabbit reticulocyte translation machinery (FIG. 4B). In separate experiments we established that wt L1 translation could also be enhanced by liver tRNA (FIG. 4), and by tRNAs extracted from bovine skin epidermis, which presumably constitutes a mixture of tRNAs from differentiated and undifferentiated cells.

Detail Description Paragraph - DETX (180):

[0238] To construct a modified gfp gene (SEQ ID NO: 11) using papillomavirus preferred codons (PGFP), 6 pairs of oligonucleotides were synthesized. Each pair of oligonucleotides has restriction sites incorporated and was used to amplify gfp using a humanized gfp gene (SEQ ID NO: 9) (GIBCO) as template. The PCR fragments were ligated into the pUC18 vector to produce pUCPGFP. The PGFP gene was sequenced, and cloned into BamHI site of the same mammalian expression vector, pCDNA3, under the CMV promoter. The DNA and deduced amino acid sequences of the humanized gfp gene are shown in FIG. 1C. Mutations introduced into the wild type gfp gene (SEQ ID NO: 9) to produce the Pgp gene (SEQ ID NO: 11) are indicated above the corresponding nucleotide residues of the wild-type sequence.

Detail Description Paragraph - DETX (182):

[0240] To further confirm that codon usage can alter gene expression in mammalian cells, we made a further variant on a synthetic gfp gene modified for optimal expression in eukaryotic cells (Zolotukhin, et al., 1996. J. Virol. 70:4646-4654). In our variant, codons optimized for expression in eukaryotic cells were substituted by those preferentially used in papillomavirus late



genes. Of 240 codons in the humanized gfp gene (SEQ ID NO: 9), which expresses high levels of fluorescent protein in cultured cells, 156 were changed to the corresponding papillomavirus late gene-preferred codons to produce a new gfp gene (SEQ ID NO: 11) designated Pgfp. Expression of Pgfp (SEQ ID NO: 11) in undifferentiated cells was compared with that of humanized gfp (SEQ ID NO: 9). COS-1 cells transfected with the humanized gfp (SEQ ID NO: 9) produced a bright fluorescent signal after 24 hrs, while cells transfected with Pgfp (SEQ ID NO: 11) produced only a faint fluorescent signal (FIG. 6A-3). To confirm that this difference reflected differing translational efficacy, gfp specific mRNA was tested in both transfections and found not to be significantly different (FIG. 6B). Thus, codon usage and corresponding tRNA availability apparently determines the observed restriction of expression of PV late genes, and modification of codon usage in other genes similarly prevents their expression in undifferentiated cells.

Detail Description Paragraph - DETX (221):

[0273] Synthetic gfp genes were constructed in which a single artificial start codon (ATG) followed by a stretch of five identical codons is fused in frame immediately upstream of a gfp coding sequence. A reverse oligonucleotide primer (SEQ ID NO: 219; sequence complementary to the termination codon for GFP, is underlined), and a suite of forward oligonucleotide primers (SEQ ID NO: 160 through 218; the first codon of GFP, is underlined) were synthesized and used for PCR amplification of a humanized gfp gene (SEQ ID NO: 158) (GIBCO) as template with Taq DNA polymerase (Amplification parameters: 95.degree. C./30 sec; 52.degree. C./30 sec; 72.degree. C./1 min; 30 cycles). The amplified fragments have nucleic acid sequences and deduced amino acid sequences as shown in SEQ ID NO: 35 through 157.

PGPUB-DOCUMENT-NUMBER: 20030175907

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175907 A1

TITLE: Method of constructing a synthetic polynucleotide

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Sun, Xiao Yi	Jindalee		AU	

APPL-NO: 10/ 305765

DATE FILED: November 27, 2002

RELATED-US-APPL-DATA:

child 10305765 A1 20021127

parent division-of 09479645 20000107 US GRANTED

parent-patent 6489141 US

child 09479645 20000107 US

parent continuation-in-part-of PCT/AU98/00530 19980709 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
AU	PO7765	1997AU-PO7765	July 9, 1997
AU	PO9467	1997AU-PO9467	September 11, 1997
AU	PP8078	1999AU-PP8078	January 8, 1999

US-CL-CURRENT: 435/91.2, 435/6

ABSTRACT:

A method of constructing a synthetic polynucleotide, the method including selecting a first codon of a parent polynucleotide that encodes a polypeptide for replacement with a synonymous codon, wherein the synonymous codon is selected on the basis that it exhibits a higher translational efficiency in an epithelial cell of a mammal than the first codon in a comparison of translational efficiencies of codons in test cells of the same type as the epithelial cell; and replacing the first codon with the synonymous codon to construct the synthetic polynucleotide.

## CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of co-pending U.S. patent application Ser. No. 09/479,645, filed Jan. 7, 2000, in the name of Ian Hector Frazer et al., which claims priority of Australian Patent Application No. PP 8078, filed Jan. 8, 1999, and which is a continuation-in-part of co-pending International Patent Application No. PCT/AU98/00530, filed Jul. 9, 1998, which designates the United States, and which claims priority of Australian Patent Application Nos. PO7765, filed Jul. 9, 1997, and PO9467, filed Sep. 11, 1997. The subject matter of both related applications PCT/AU98/00530 and 09/479,645 are hereby incorporated herein by reference.

----- KWIC -----

### Brief Description of Drawings Paragraph - DRTX (14):

[0061] FIG. 6A, comprising FIGS. 6A-1, 6A-2, and 6A-3, is a series of images, each of which depicts a confocal micrograph showing expression of GFP in COS-1 cells transfected with wild-type gfp (wt) (FIG. 6A-2) or a synthetic gfp gene (FIG. 6A-3) carrying codons used at relatively high frequency by papillomavirus genes (p). FIG. 6A-1 shows results from a mock transfection.

### Brief Description of Drawings Paragraph - DRTX (16):

[0063] FIG. 7, comprising FIGS. 7A through 7L, is a series of images, each of which depicts the expression pattern of GFP in vivo from wild-type gfp gene, or a synthetic gfp gene carrying codons used at relatively high frequency by papillomavirus genes. Using a gene gun, mice were shot with PGFP (FIGS. 7A through 7F) and GFP (FIGS. 7G through 7L) expression plasmids encoding GFP protein. A transverse section of the mouse skin section shows where the gfp gene is expressed. Bright-field photographs (FIGS. 7A, 7B, 7G, and 7H) of the same section where dermis (D) epidermis (E) are highlighted are shown to identify the location of fluorescence in the epidermis. Arrows indicate fluorescent signals.

### Detail Description Paragraph - DETX (171):

[0228] As the major limitation to expression of the wild type BPV L1 and L2 genes appeared to be translational in our system, we wished to test whether this limitation reflected a limited availability of the appropriate tRNA species for gene translation. As transient expression of the synthetic genes within intact cells can be regulated by many factors, we tested our hypothesis in a cell free system using rabbit reticulocyte lysate (RRL) or wheat germ lysate to examine gene translation. Similar amounts of plasmids expressing the wild type or synthetic humanized BPV1 L1 gene were added to a T7-DNA polymerase coupled RRL transcription/translation system in the presence of 35S-methionine. After 20 minutes, translated proteins were separated by SDS PAGE and visualized by autoradiography. Efficient translation of the modified L1 gene was observed (FIG. 4A, lane 2), while translation of the wild type BPV1 L1 sequence resulted in a weak 55 kDa L1 band (FIG. 4A, lane 1). We reasoned that although the wild type sequence was not optimized for translation in RRL, some translation would

occur, as there would be no cellular mRNA species competing for the 'rare' codons present in the wild type L1 sequence. The above data suggest that the observed difference in efficiency of translation of the wild type and synthetic humanized L1 genes is a consequence of limited availability of the tRNAs required for translation of the rare codons present in the wild type gene. We therefore expected that addition of excess tRNA to the in vitro translation system would overcome the inhibition of translation of the wild type L1 gene. To address this question, 10-5 M aminoacyl-tRNAs from yeast were added into the RRL translation system, and L1 protein synthesis was assessed. Introduction of exogenous tRNAs resulted in a dramatic improvement in translation of the wild type L1 sequence, which now gave a yield of L1 protein comparable to that observed with the synthetic humanized L1 sequence (SEQ ID NO: 3) (FIG. 4A). Enhancement of translation of the wild type L1 gene (SEQ ID NO: 1) by aminoacyl-tRNA was dose-dependent, with an optimum efficiency at 10-5 M tRNA. As addition of exogenous tRNA improved the yield of L1 protein translated from the wild type L1 gene sequence (SEQ ID NO: 1), we assessed the speed of translation of wild type and humanized L1 mRNA. Samples were collected from the translation mixture every 2 minutes, starting at the 8th minute. Translation of L1 (SEQ ID NO: 2, 4) from the wild type sequence (SEQ ID NO: 1) was much slower than from the humanized L1 sequence (SEQ ID NO: 3) (FIG. 4B), and the retardation of translation could be completely overcome by adding exogenous tRNA from commercially available yeast tRNA. Yeast tRNA was chosen in the above analysis because the codon usage in yeast is similar to that of papillomavirus (Table 1). Addition of exogenous tRNA did not significantly improve the translation of the humanized L1 gene (SEQ ID NO: 3), indicating that this sequence was optimized with regard to codon usage for the rabbit reticulocyte translation machinery (FIG. 4B). In separate experiments we established that wt L1 translation could also be enhanced by liver tRNA (FIG. 4), and by tRNAs extracted from bovine skin epidermis, which presumably constitutes a mixture of tRNAs from differentiated and undifferentiated cells.

Detail Description Paragraph - DETX (182):

[0233] To construct a modified gfp gene (SEQ ID NO: 11) using papillomavirus preferred codons (PGFP), 6 pairs of oligonucleotides were synthesized. Each pair of oligonucleotides has restriction sites incorporated and was used to amplify gfp using a humanized gfp gene (SEQ ID NO: 9) (GIBCO) as template. The PCR fragments were ligated into the pUC18 vector to produce pUCPGFP. The PGFP gene was sequenced, and cloned into BamHI site of the same mammalian expression vector, pCDNA3, under the CMV promoter. The DNA and deduced amino acid sequences of the humanized gfp gene are shown in FIG. 1C. Mutations introduced into the wild type gfp gene (SEQ ID NO: 9) to produce the Pgf gene (SEQ ID NO: 11) are indicated above the corresponding nucleotide residues of the wild-type sequence.

Detail Description Paragraph - DETX (184):

[0235] To further confirm that codon usage can alter gene expression in mammalian cells, we made a further variant on a synthetic gfp gene modified for optimal expression in eukaryotic cells (Zolotukhin, et al., 1996. J. Virol. 70:4646-4654). In our variant, codons optimized for expression in eukaryotic cells were substituted by those preferentially used in papillomavirus late

genes. Of 240 codons in the humanized gfp gene (SEQ ID NO: 9), which expresses high levels of fluorescent protein in cultured cells, 156 were changed to the corresponding papillomavirus late gene-preferred codons to produce a new gfp gene (SEQ ID NO: 11) designated Pgfp. Expression of Pgfp (SEQ ID NO: 11) in undifferentiated cells was compared with that of humanized gfp (SEQ ID NO: 9). COS-1 cells transfected with the humanized gfp (SEQ ID NO: 9) produced a bright fluorescent signal after 24 hrs, while cells transfected with Pgfp (SEQ ID NO: 11) produced only a faint fluorescent signal (FIG. 6A-3). To confirm that this difference reflected differing translational efficacy, gfp specific mRNA was tested in both transfections and found not to be significantly different (FIG. 6B). Thus, codon usage and corresponding tRNA availability apparently determines the observed restriction of expression of PV late genes, and modification of codon usage in other genes similarly prevents their expression in undifferentiated cells.

Detail Description Paragraph - DETX (222):

[0262] Synthetic gfp genes were constructed in which a single artificial start codon (ATG) followed by a stretch of five identical codons is fused in frame immediately upstream of a gfp coding sequence. A reverse oligonucleotide primer (SEQ ID NO: 219; sequence complementary to the termination codon for GFP, is underlined), and a suite of forward oligonucleotide primers (SEQ ID NO: 160 through 218; the first codon of GFP, is underlined) were synthesized and used for PCR amplification of a humanized go gene (SEQ ID NO: 158) (GIBCO) as template with Taq DNA polymerase (Amplification parameters: 95.degree. C./30 sec; 52.degree. C./30 sec; 72.degree. C./1 min; 30 cycles). The amplified fragments have nucleic acid sequences and deduced amino acid sequences as shown in SEQ ID NO: 35 through 157.

PGPUB-DOCUMENT-NUMBER: 20030170293

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030170293 A1

TITLE: Thermotolerant phytase for animal feed

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Betts, Scott	Durham	NC	US	

APPL-NO: 10/ 334671

DATE FILED: December 30, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60344476 20011228 US

US-CL-CURRENT: 424/442, 435/196

ABSTRACT:

The invention provides a synthetic phytase polynucleotide which is optimized for expression in plants and which encodes a thermotolerant phytase, as well as isolated thermotolerant phytase enzyme. Also provided are feed or food products comprising a thermotolerant phytase, and transgenic plants which express the thermotolerant phytase. Further provided are methods for making and using thermotolerant phytases, e.g., a method of using a thermotolerant phytase in feed and food processing.

RELATED APPLICATION

[0001] This application claims priority to Application No. 60/344,476, filed Dec. 28, 2001, which is incorporated by reference herein.

----- KWIC -----

Detail Description Paragraph - DETX (47):

[0099] "**Regulated** promoter" refers to promoters that direct gene expression not constitutively, but in a temporally- and/or spatially-**regulated** manner, and include both tissue-specific and inducible promoters. It includes natural and **synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences**. Different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of

development, or in response to different environmental conditions. New promoters of various types useful in plant cells are constantly being discovered, numerous examples may be found in the compilation by Okamuro et al. (1989). Typical regulated promoters useful in plants include but are not limited to safener-inducible promoters, promoters derived from the tetracycline-inducible system, promoters derived from salicylate-inducible systems, promoters derived from alcohol-inducible systems, promoters derived from glucocorticoid-inducible system, promoters derived from pathogen-inducible systems, and promoters derived from ecdysome-inducible systems.

Detail Description Paragraph - DETX (48):

[0100] "Regulatory sequences" and "suitable regulatory sequences" each refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences include enhancers, promoters, translation leader sequences, introns, and polyadenylation signal sequences. They include natural and synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences. As is noted above, the term "suitable regulatory sequences" is not limited to promoters. Some suitable regulatory sequences useful with plants in the present invention will include, but are not limited to constitutive plant promoters, plant tissue-specific promoters, plant development-specific promoters, inducible plant promoters and viral promoters.

Detail Description Paragraph - DETX (67):

[0119] The nucleic acid molecules of the invention are optimized for enhanced expression in an organism of interest. For plants, see, for example, EPA035472; WO 91/16432; Perlak et al., 1991; and Murray et al., 1989. In this manner, the genes or gene fragments can be synthesized utilizing plant-preferred codons. See, for example, Campbell and Gowri, 1990 for a discussion of host-preferred codon usage. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, synthetic or partially optimized sequences may also be used. Variant nucleotide sequences and proteins also encompass sequences and protein derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more different coding sequences can be manipulated to create a new polypeptide possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined in vitro or in vivo. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer, 1994; Stemmer, 1994; Cramer et al., 1997; Moore et al., 1997; Zhang et al., 1997; Cramer et al., 1998; and U.S. Pat. Nos. 5,605,793 and 5,837,458.

Detail Description Paragraph - DETX (89):

[0141] The regulatory sequences may be located upstream (5' non-coding sequences), within (intron), or downstream (3' non-coding sequences) of a coding sequence, and influence the transcription, RNA processing or stability,

and/or translation of the associated coding sequence. **Regulatory** sequences may include, but are not limited to, enhancers, promoters, repressor binding sites, translation leader sequences, introns, and polyadenylation signal sequences. They may include natural and **synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences.**

Detail Description Paragraph - DETX (228):

[0278] Induction of expression of a nucleic acid sequence of the present invention using systems based on steroid hormones is also contemplated. For example, a glucocorticoid-mediated induction system is used (Aoyama and Chua (1997) The Plant Journal 11: 605-612) and **gene expression is induced by application of a glucocorticoid, such as a synthetic** glucocorticoid, preferably dexamethasone, preferably at a concentration ranging from 0.1 mM to 1 mM, more preferably from 10 nM to 10 mM. For the purposes of the present invention, the **luciferase** gene sequences are replaced by a nucleic acid sequence of the invention to form an expression cassette having a nucleic acid sequence of the invention under the control of six copies of the GAL4 upstream activating sequences fused to the 35S minimal promoter. This is carried out using methods well known in the art. The trans-acting factor comprises the GAL4 DNA-binding domain (Keegan et al. (1986) Science 231: 699-704) fused to the transactivating domain of the herpes viral protein VP16 (Triezenberg et al. (1988) Genes Devel. 2: 718-729) fused to the hormone-binding domain of the rat glucocorticoid receptor (Picard et al. (1988) Cell 54: 1073-1080). The expression of the fusion protein is controlled by any promoter suitable for expression in plants known in the art or described here. This expression cassette is also comprised in the plant comprising a nucleic acid sequence of the invention fused to the 6xGAL4/minimal promoter. Thus, tissue- or organ-specificity of the fusion protein is achieved leading to inducible tissue- or organ-specificity of the expression cassettes of the present invention.



PGPUB-DOCUMENT-NUMBER: 20030159161

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030159161 A1

TITLE: Synthetic genes and genetic constructs comprising same  
I

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Graham, Michael Wayne	Jindalee		AU	
Rice, Robert Norman	Sinmonon Park		AU	

APPL-NO: 10/ 346853

DATE FILED: January 17, 2003

RELATED-US-APPL-DATA:

child 10346853 A1 20030117

parent continuation-of 09100812 19980619 US GRANTED

parent-patent 6573099 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
AU	PP2292	1998AU-PP2292	March 20, 1998

US-CL-CURRENT: 800/8, 435/320.1 , 435/325 , 435/455

ABSTRACT:

The present invention relates generally to synthetic genes for modifying endogenous gene expression in a cell, tissue or organ of a transgenic organism, in particular a transgenic animal or plant. More particularly, the present invention provides novel synthetic genes and genetic constructs which are capable of repressing delaying or otherwise reducing the expression of an endogenous gene or a target gene in an organism when introduced thereto.

----- KWIC -----

Summary of Invention Paragraph - BSTX (7):

[0005] In work leading up to the present invention, the inventors sought to elucidate the mechanisms involved in down-regulating gene expression in an attempt to provide improved methods therefor. In so doing the inventors have

developed a wide range of synthetic genes capable of modulating gene expression in both prokaryotic and eukaryotic cells and genetic constructs comprising same.

Detail Description Paragraph - DETX (8):

[0049] The term "synthetic gene" refers to a non-naturally occurring gene as hereinbefore defined which preferably comprises at least one or more transcriptional and/or translational regulatory sequences operably linked to a structural gene sequence.

Detail Description Paragraph - DETX (16):

[0057] Generally, a gene of the invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions without affecting its ability to modify target gene expression. Nucleotide insertional derivatives of the synthetic gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product.

Detail Description Paragraph - DETX (26):

[0067] Alternatively, the structural gene may comprise a nucleotide sequence which does not encode an amino acid sequence or more commonly, comprises one or more open reading frames which encode one or more peptides, oligopeptides or polypeptides which are unrelated at the amino acid sequence level to the amino acid sequence encoded by the target gene. For example, the mRNA product of the structural gene may be inserted into the synthetic gene of the invention so as to alter or disrupt the reading frame of the structural gene and produce one or more frame shift mutations in the translation product thereof relative to the translation product encoded by the target gene, notwithstanding a substantial identity between the structural gene and the target gene on the one hand and the corresponding mRNA products of the structural gene and the target gene on the other hand. Such effects may be produced by introducing one or two nucleotide substitutions or deletions into the structural gene, relative to the target gene sequence or alternatively, by introducing a translation start codon 5'-ATG-3' upstream of any nucleotide in the structural gene which occurs at a particular position in a codon of the corresponding target gene such that the position of said nucleotide in the codon of the structural gene is altered.

Detail Description Paragraph - DETX (30):

[0071] A promoter is usually, but not necessarily, positioned upstream or 5', of the structural gene component of the synthetic gene of the invention, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the structural gene.

Detail Description Paragraph - DETX (33):

[0074] Examples of promoters suitable for use in the synthetic genes of the present invention include viral, fungal, bacterial, animal and plant derived promoters capable of functioning in plant, animal, insect, fungal, yeast or bacterial cells. The promoter may regulate the expression of the structural gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or pathogens, or metal ions, amongst others.

Detail Description Paragraph - DETX (172):

Synthetic Genes and Genetic Constructs Comprising the lacI and Green Fluorescent Protein (GFP) Open Reading Frames

Detail Description Paragraph - DETX (177):

Synthetic Genes and Genetic Constructs Comprising the lacI and Green Fluorescent Protein (GFP) and Tyrosinase Open Reading Frames

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ABSTRACT:

A synthetic nucleic acid molecule is provided that includes nucleotides of a coding region for a fluorescent polypeptide having a codon composition differing at more than 25% of the codons from a parent nucleic acid sequence encoding a fluorescent polypeptide. The synthetic nucleic acid molecule has at least 3-fold fewer transcription regulatory sequences relative to the average number of such sequences in the parent nucleic acid sequence. The polypeptide encoded by the synthetic nucleic acid molecule preferably has at least 85% sequence identity to the polypeptide encoded by the parent nucleic acid sequence.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn.120 to U.S. patent application Ser. No. 09/645,706, filed Aug. 24, 2000, the entirety of which is incorporated by reference herein.

----- KWIC -----

Abstract Paragraph - ABTX (1):

A synthetic nucleic acid molecule is provided that includes nucleotides of a coding region for a fluorescent polypeptide having a codon composition differing at more than 25% of the codons from a parent nucleic acid sequence encoding a fluorescent polypeptide. The synthetic nucleic acid molecule has at least 3-fold fewer transcription regulatory sequences relative to the average number of such sequences in the parent nucleic acid sequence. The polypeptide encoded by the synthetic nucleic acid molecule preferably has at least 85% sequence identity to the polypeptide encoded by the parent nucleic acid sequence.

Summary of Invention Paragraph - BSTX (9):

[0007] However, altering codon usage may, in turn, result in the unintentional introduction into a synthetic nucleic acid molecule of inappropriate transcription regulatory sequences. This may adversely effect transcription, resulting in anomalous expression of the synthetic DNA. Anomalous expression is defined as departure from normal or expected levels of expression. For example, transcription factor binding sites located downstream from a promoter have been demonstrated to effect promoter activity (Michael et al., 1990; Lamb et al., 1998; Johnson et al., 1998; Jones et al., 1997). Additionally, it is not uncommon for an enhancer sequence to exert activity and result in elevated levels of DNA transcription in the absence of a promoter or for the presence of transcription regulatory sequences to increase the basal levels of gene expression in the absence of a promoter.

Summary of Invention Paragraph - BSTX (13):

[0010] The invention, which is defined by the claims set out at the end of this disclosure, is intended to solve at least some of the problems noted above. The invention provides a synthetic nucleic acid molecule comprising nucleotides of a coding region for a fluorescent polypeptide having a codon composition differing at more than 25% of the codons from a parent nucleic acid sequence encoding a fluorescent polypeptide and having at least 3-fold fewer transcription regulatory sequences relative to the average number of such sequences in the parent nucleic acid sequence. Preferably, the synthetic nucleic acid molecule encodes a polypeptide that has an amino acid sequence that is at least 85%, preferably at least 90%, and most preferably at least 95% or at least 99% identical to the amino acid sequence of the parent (parent or another synthetic) polypeptide (protein) from which it is derived. Thus, it is recognized that some specific amino acid changes may also be desirable to alter a particular phenotypic characteristic of the polypeptide encoded by the synthetic nucleic acid molecule. Preferably, the amino acid sequence identity is over at least 100 contiguous amino acid residues. In one embodiment of the invention, the codons in the synthetic nucleic acid molecule that differ preferably encode the same amino acids as the corresponding codons in the parent nucleic acid sequence.

Summary of Invention Paragraph - BSTX (14):

[0011] The transcription regulatory sequences that are reduced in the synthetic nucleic acid molecule include, but are not limited to, any combination of transcription factor binding sequences, intron splice sequences,

poly(A) addition sequences, enhancer sequences and promoter sequences. Transcription **regulatory** sequences are well known in the art. It is preferred that the synthetic nucleic acid molecule of the invention has a codon composition that differs from that of the parent nucleic acid sequence at more than 25%, 30%, 35%, 40% or more than 45%, e.g., 50%, 55%, 60% or more of the codons. Codons for use in the invention are those which are employed more frequently than at least one other codon for the same amino acid in a particular organism and, more preferably, are also not low-usage codons in that organism and are not low-usage codons in the organism, for example, *E. coli*, used to clone or screen for the expression of the synthetic nucleic acid molecule. Moreover, preferred codons for certain amino acids, i.e., those amino acids that have three or more codons, may include two or more codons that are employed more frequently than the other (non-preferred) codon(s). The presence of codons in the synthetic nucleic acid molecule that are employed more frequently in one organism than in another organism results in a synthetic nucleic acid molecule which, when introduced into the cells of the organism that employs those codons more frequently, is expressed in those cells at a level that is greater than the expression of the parent nucleic acid sequence in those cells. For example, the synthetic nucleic acid molecule of the invention is expressed at a level that is at least about 105%, e.g., 110%, 150%, 200%, 500% or more (e.g., 1000%, 5000%, or 10000%), of that of the parent nucleic acid sequence in a cell or cell extract under identical conditions (such as cell culture conditions, vector backbone, and the like).

#### Summary of Invention Paragraph - BSTX (24):

[0021] The invention also provides a method to prepare a synthetic nucleic acid molecule of the invention by genetically altering a parent (either wild type or another **synthetic nucleic acid sequence**). The method may be used to prepare a synthetic nucleic acid molecule encoding a fluorescent protein. The method of the invention may be employed to alter the codon usage frequency and decrease the number of transcription **regulatory** sequences in an open reading frame of any protein (e.g., a fluorescent protein) or to decrease the number of transcription **regulatory** sites in a vector backbone. Preferably, the codon usage frequency in the synthetic nucleic acid molecule is altered to reflect that of the host organism desired for expression of that nucleic acid molecule while also decreasing the number of potential transcription **regulatory** sequences relative to the parent nucleic acid molecule.

#### Summary of Invention Paragraph - BSTX (25):

[0022] Thus, the invention provides a method to prepare a synthetic nucleic acid molecule comprising an open reading frame. The method comprises altering a plurality of transcription **regulatory** sequences in a parent nucleic acid **sequence which encodes a fluorescent polypeptide to yield a synthetic** nucleic acid molecule which has at least 3-fold fewer transcription **regulatory** sequences relative to the parent nucleic acid sequence. The method also comprises altering greater than 25% of the codons in the **synthetic nucleic acid sequence which has a decreased number of transcription regulatory sequences to yield a further synthetic** nucleic acid molecule. The codons which are altered do not result in an increased number of transcription **regulatory** sequences. The further synthetic nucleic acid molecule encodes a polypeptide with at least

85% amino acid sequence identity to the polypeptide encoded by the parent nucleic acid sequence.

Summary of Invention Paragraph - BSTX (26):

[0023] Alternatively, the method comprises altering greater than 25% of the codons in a parent nucleic acid sequence which encodes a fluorescent polypeptide to yield a codon-altered synthetic nucleic acid molecule. The method also comprises altering a plurality of transcription regulatory sequences in the codon-altered synthetic nucleic acid molecule to yield a further synthetic nucleic acid molecule which has at least 3-fold fewer transcription regulatory sequences relative to a synthetic nucleic acid molecule with codons which differ from the corresponding codons in the parent nucleic acid sequence. The further synthetic nucleic acid molecule encodes a polypeptide with at least 85% amino acid sequence identity to the fluorescent polypeptide encoded by the parent nucleic acid sequence.

Summary of Invention Paragraph - BSTX (29):

[0026] The invention also provides at least two synthetic nucleic acid molecules that encode highly related polypeptides, but which synthetic nucleic acid molecules have an increased number of nucleotide differences relative to each other. These differences decrease the recombination frequency between the two synthetic nucleic acid molecules when those molecules are both present in a cell (i.e., they are "codon distinct" versions of a synthetic nucleic acid molecule). Thus, the invention provides a method for preparing at least two synthetic nucleic acid molecules that are codon distinct versions of a parent nucleic acid sequence that encodes a polypeptide. The method comprises altering a parent nucleic acid sequence to yield a first synthetic nucleic acid molecule having an increased number of a first plurality of codons that are employed more frequently in a selected host cell relative to the number of those codons present in the parent nucleic acid sequence. Optionally, the first synthetic nucleic acid molecule also has a decreased number of transcription regulatory sequences relative to the parent nucleic acid sequence. The parent nucleic acid sequence is also altered to yield a second synthetic nucleic acid molecule having an increased number of a second plurality of codons that are employed more frequently in the host cell relative to the number of those codons in the parent nucleic acid sequence. The first plurality of codons is different than the second plurality of codons. The first and the second synthetic nucleic acid molecules preferably encode the same polypeptide. Optionally, the second synthetic nucleic acid molecule has a decreased number of transcription regulatory sequences relative to the parent nucleic acid sequence. Either or both synthetic molecules can then be further modified.

Detail Description Paragraph - DETX (24):

[0059] The terms "cell," "cell line," "host cell," as used herein, are used interchangeably, and all such designations include progeny or potential progeny of these designations. By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced a DNA molecule. Optionally, a synthetic gene of the invention may be introduced into a suitable cell line so

as to create a transfected ("stably" or "transient") cell line capable of producing the protein or polypeptide encoded by the synthetic gene. Vectors, cells, and methods for constructing such cell lines are well known in the art, e.g. in Ausubel, et al (1992). The words "transformants" or "transformed cells" include the primary transformed cells derived from the originally transformed cell without regard to the number of transfers. All progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Nonetheless, mutant progeny that have the same functionality as screened for in the originally transformed cell are included in the definition of transformants.

Detail Description Paragraph - DETX (80):

[0115] To select codons for the synthetic nucleic acid molecules of the invention, preferred codons have a relatively high codon usage frequency in a selected host cell, and their introduction results in the introduction of relatively few transcription factor binding sequences, relatively few other undesirable structural attributes, and optionally a characteristic that distinguishes the synthetic gene from another gene encoding a highly similar protein. Thus, the synthetic nucleic acid product obtained by the method of the invention is a synthetic gene with improved level of expression due to improved codon usage frequency, a reduced risk of inappropriate transcriptional behavior due to a reduced number of undesirable transcription regulatory sequences, and optionally any additional characteristic due to other criteria that may be employed to select the synthetic sequence.

Detail Description Paragraph - DETX (81):

[0116] Optimally, at least one characteristic in the synthetic gene is enhanced protein expression in the desired host cell vis-a-vis the native host cell. Thus, the synthetic nucleic acid product obtained by the method of the invention is a synthetic fluorescent protein gene with improved level of expression due to improved codon usage, a reduced risk of inappropriate transcriptional behavior due to a reduced number of undesirable transcription regulatory sequences, and optionally any additional characteristic due to other criteria that may be employed to select the synthetic sequence.

Detail Description Paragraph - DETX (86):

[0121] Thus, generally, the method of the invention comprises identifying a target nucleic acid sequence that encodes a fluorescent protein, and a host cell of interest, for example, a plant (dicot or monocot), fungus, yeast, or mammalian cell. Preferred host cells are mammalian host cells such as CHO, COS, 293, HeLa, CV-1 and NIH3T3 cells. Based on preferred codon usage in the host cell(s) and, optionally, low codon usage in the host cell(s), e.g., high usage mammalian codons and low usage E. coli and mammalian codons, codons to be replaced are determined. Codon distinct versions of two synthetic nucleic acid molecules may be determined using alternative preferred codons are introduced to each version. Thus, for amino acids having more than two codons, one preferred codon is introduced to one version and another preferred codon is introduced to the other version. For amino acids having more than one codon, the two codons with the largest number of mismatched bases may be identified



and one is introduced to one version and the other codon is introduced to the other version. Concurrent, subsequent, or prior to selecting codons to be replaced, desired and undesired sequences, such as undesired transcriptional regulatory sequences, in the target sequence are identified. These sequences can be identified using databases and software such as EPD, NNPD, REBASE, TRANSFAC, TESS, GenePro, MAR ([www.ncgr.org/MAR-search](http://www.ncgr.org/MAR-search)) and BCM Gene Finder, further described herein. After the sequences are identified, the modification(s) are introduced. Once a desired synthetic nucleic acid sequence is obtained, it can be prepared by methods well known to the art (such as PCR with overlapping primers or commercial gene synthesis), and its structural and functional properties compared to the target nucleic acid sequence, including, but not limited to, percent identity, presence or absence of certain sequences, for example, restriction sequences, percent of codons changed (such as an increased or decreased usage of certain codons) and expression rates.

Detail Description Paragraph - DETX (95):

[0130] As described below, the method was used to create a synthetic gene encoding a green fluorescent protein (GFP) that was a mutated form of a GFP originally isolated from *Montastraea cavernosa*. The synthetic gene supports much greater levels of fluorescence in a host cell when compared to the parent GFP. In addition, it is expected that there will be decreased anomalous expression of the synthetic GFP when compared to the parent GFP.

Detail Description Paragraph - DETX (97):

[0132] The synthetic genes of the invention preferably encode the same proteins as their parental counterpart (or nearly so), and, when compared to the parent protein, have improved codon usage while being largely devoid of known transcription regulatory sequences in the coding region. (It is recognized that a small number of amino acid changes may be desired to enhance a property of the native counterpart protein, e.g. to enhance the fluorescent properties of a fluorescent protein.) This increases the level of expression of the protein encoded by the synthetic gene and reduces the risk of anomalous expression of the protein. For example, studies of many important events of gene regulation, which may be mediated by weak promoters, are limited by insufficient reporter signals from inadequate expression of the reporter proteins. The synthetic fluorescent protein genes described herein permit detection of weak promoter activity because of the large increase in level of expression, which enables increased detection sensitivity. A further benefit is that transcription factors that may be available in limited quantities are not utilized by the cell in non-productive binding events. Also, the use of some selectable markers may be limited by the expression of that marker in an exogenous cell. Thus, synthetic selectable marker genes which have improved codon usage for that cell, and have a decrease in other undesirable sequences, (e.g., transcription factor binding sequences), can permit the use of those markers in cells that otherwise were undesirable as hosts for those markers.

Detail Description Paragraph - DETX (98):

[0133] Promoter crosstalk is another concern when a co-reporter gene is used to normalize transfection efficiencies. With the enhanced expression of

synthetic genes, the amount of DNA containing strong promoters can be reduced, or DNA containing weaker promoters can be employed, to drive the expression of the co-reporter. In addition, there may be a reduction in the background expression from the synthetic reporter genes of the invention. This characteristic makes synthetic reporter genes more desirable by minimizing the sporadic expression from the genes and reducing the interference resulting from other regulatory pathways.

Detail Description Paragraph - DETX (99):

[0134] The use of reporter genes in imaging systems, which can be used for in vivo biological studies or drug screening, is another use for the synthetic genes of the invention. Due to their increased level of expression, the protein encoded by a synthetic gene is more readily detectable by an imaging system. In the case of a fluorescent protein encoded by a synthetic gene, during fluorescence activated cell sorting (FACS), fluorescence intensity may be increased or reduced, according to need of the investigator. In addition, the synthetic fluorescent protein genes may be used to express fusion proteins, for example fusions with secretion leader sequences or cellular localization sequences, to study transcription in difficult-to-transfect cells such as primary cells, and/or to improve the analysis of regulatory pathways and genetic elements. Further, synthetic fluorescent protein genes may be fused to a gene of interest such that expression of the gene of interest can be tracked, e.g., inside a host cell.

Detail Description Paragraph - DETX (100):

[0135] Other uses include, but are not limited to, the detection of rare events that require extreme sensitivity (e.g., studying RNA recoding), use with internal ribosome entry sites (IRES), to improve the efficiency of in vitro translation or in vitro transcription-translation coupled systems such as TNT.TM. (Promega Corp., Madison, Wis.), study of fluorescent proteins optimized to different host organisms (e.g., plants, fungi, and the like). In addition, the synthetic fluorescent proteins of the invention can be used as reporters. Thus, the fluorescent proteins can be used as reporter molecules in multiwell assays, and as reporter molecules in drug screening with the advantage of minimizing possible interference of reporter signal by different signal transduction pathways and other regulatory mechanisms. Multiple synthetic fluorescent protein genes can be used as co-reporters to, e.g., monitor drug toxicity.

Detail Description Paragraph - DETX (108):

[0141] Green II was used as a parent gene in humanization of the nucleic acid sequences. A synthetic gene sequence was designed in silico using the following software tools: MatInspector professional Release 5.2 with Matrix Family Library Ver 2.3 and 2.4, ModelInspector professional Release 4.7.8 and 4.7.9 with Promoter Module Library Ver 2.2 and 2.3, and SequenceShaper Release 2.3 (all from Genomatix Software GmbH, Munich, Germany). The gene was designed to 1) have optimized codon usage for expression in mammalian cells, 2) have a reduced number of transcriptional regulatory sequences including vertebrate transcription factor binding sequences, splice sequences, poly(A) addition

sequences and promoter sequences, as well as prokaryotic (e.g., E. coli) **regulatory** sequences, 3) have a Kozak sequence, 4) have at least one novel restriction enzyme recognition sequence for cloning, and 5) be devoid of unwanted restriction enzyme recognition sequences, e.g., those which are likely to interfere with standard cloning procedures.

Detail Description Paragraph - DETX (109):

[0142] Not all design criteria could be met equally well at the same time. The following priority was established: elimination of vertebrate transcription factor (TF) binding sequences received the highest priority, followed by elimination of splice sequences and poly(A) addition sequences, and finally elimination of prokaryotic **regulatory** sequences. When removing **regulatory** sequences, the strategy was to work from the lesser important to the most important to ensure that the most important changes were made last, and inadvertent changes to these improvements did not occur. Then the sequence was rechecked for the appearance of new lower priority sequences and additional changes made as needed. Thus, the process for designing a **synthetic gene** sequence, using computer programs described herein, involves optionally iterative steps that are detailed below.

Claims Text - CLTX (2):

1. A synthetic nucleic acid molecule comprising nucleotides of a coding region for a fluorescent polypeptide having a codon composition differing at more than 25% of the codons from a parent nucleic acid **sequence encoding a fluorescent polypeptide, wherein the synthetic** nucleic acid molecule has at least 3-fold fewer transcription **regulatory** sequences relative to the average number of such sequences in the parent nucleic acid sequence.

Claims Text - CLTX (3):

2. The **synthetic nucleic acid molecule of claim 1, wherein the transcription regulatory sequences** are selected from the group consisting of transcription factor binding sequences, intron splice sequences, poly(A) addition sequences, and promoter sequences.

Claims Text - CLTX (52):

51. A method to prepare a synthetic nucleic acid molecule comprising an open reading frame, comprising: a) altering a plurality of transcription **regulatory** sequences in a parent nucleic acid **sequence which encodes a fluorescent polypeptide to yield a synthetic** nucleic acid molecule which has at least 3-fold fewer transcription **regulatory** sequences relative to the parent nucleic acid sequence; and b) altering greater than 25% of the codons in the **synthetic nucleic acid sequence which has a decreased number of transcription regulatory sequences to yield a further synthetic** nucleic acid molecule, wherein the codons which are altered do not result in an increased number of transcription **regulatory sequences, wherein the further synthetic** nucleic acid molecule encodes a polypeptide with at least 85% amino acid sequence identity to the polypeptide encoded by the parent nucleic acid sequence.

Claims Text - CLTX (53):

52. A method to prepare a synthetic nucleic acid molecule comprising an open reading frame, comprising: a) altering greater than 25% of the codons in a parent nucleic acid sequence which encodes a fluorescent polypeptide to yield a codon-altered synthetic nucleic acid molecule, and b) altering a plurality of transcription regulatory sequences in the codon-altered synthetic nucleic acid molecule to yield a further synthetic nucleic acid molecule which has at least 3-fold fewer transcription regulatory sequences relative to a synthetic nucleic acid molecule with codons which differ from the corresponding codons in the parent nucleic acid sequence, and wherein the further synthetic nucleic acid molecule encodes a polypeptide with at least 85% amino acid sequence identity to the fluorescent polypeptide encoded by the parent nucleic acid sequence.

Claims Text - CLTX (63):

62. The method of claim 61, further comprising altering a plurality of transcription regulatory sequences in the synthetic nucleic acid molecule, the further synthetic nucleic acid molecule, or both, to yield at least one yet further synthetic nucleic acid molecule which has at least 3-fold fewer transcription regulatory sequences relative to the synthetic nucleic acid molecule, the further synthetic nucleic acid molecule, or both.

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ABSTRACT:

The invention relates to compositions comprising a first fusion protein comprising a first polypeptide domain and a R. reniformis luciferase and a second fusion protein comprising a second polypeptide domain and a R. reniformis GFP. The invention also relates to compositions comprising one or more polynucleotides encoding a first fusion protein comprising a first polypeptide domain and a R. reniformis luciferase and a second fusion protein comprising a second polypeptide domain and a R. reniformis GFP. The invention also relates to methods and kits for detecting protein-protein interactions, determining the location of a protein-protein interaction, identifying cells wherein there is a protein-protein interaction of interest, and screening for a candidate modulator that increases or decreases the amount of a protein-protein interaction.

RELATED APPLICATION(S)

[0001] This application claims the benefit of U.S. Provisional Application No. 60/329354, filed on Oct. 15, 2001. The entire teachings of the above application is incorporated herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX (12):

[0010] In one embodiment, the polynucleotide sequence of R. reniformis GFP is humanized.

Summary of Invention Paragraph - BSTX (16):

[0014] In one embodiment, the fused heterologous polypeptide domain is fused to the amino-terminal end of the R. reniformis GFP or variant thereof, wherein R. reniformis GFP is encoded by a humanized GFP polynucleotide sequence.

Summary of Invention Paragraph - BSTX (18):

[0016] In another embodiment, the fused heterologous polypeptide domain is fused to the carboxy-terminal end of the R. reniformis GFP or variant thereof, wherein R. reniformis GFP is encoded by a humanized GFP polynucleotide sequence.

Summary of Invention Paragraph - BSTX (20):

[0018] In another embodiment, the fused heterologous polypeptide domain is fused to the R. reniformis GFP or variant thereof via a linker sequence, wherein R. reniformis GFP is encoded by a humanized GFP polynucleotide sequence.

Summary of Invention Paragraph - BSTX (23):

[0021] In one embodiment, the R. reniformis GFP polypeptide is encoded by a humanized polynucleotide sequence.

Summary of Invention Paragraph - BSTX (30):

[0028] In one embodiment, the R. reniformis GFP polypeptide is encoded by a humanized polynucleotide sequence.

Summary of Invention Paragraph - BSTX (35):

[0033] In one embodiment, the R. reniformis GFP polypeptide is encoded by a humanized polynucleotide sequence.

Summary of Invention Paragraph - BSTX (43):

[0041] In one embodiment, R. reniformis GFP polypeptide is encoded by a humanized polynucleotide sequence.

Summary of Invention Paragraph - BSTX (55):

[0053] In another embodiment, the R. reniformis GFP polypeptide is encoded by a humanized polynucleotide sequence.

Summary of Invention Paragraph - BSTX (69):

[0067] As used herein, "humanized GFP" refers to a Renilla reniformis GFP polynucleotide coding sequence in which one or more, (for example, 2, 3, 4, 5, 10, 20, 50, 75, 100, 200, 500 or more, including, in certain embodiments, all the codons of the polynucleotide coding sequence for the non-human GFP polypeptide (i.e., a polypeptide not naturally expressed in humans) have been altered to a codon sequence more preferred for expression in human cells.

Summary of Invention Paragraph - BSTX (70):

[0068] A polypeptide coding sequence is herein referred to as "humanized" if one or more codons is altered from the natural coding sequence to a codon which is utilized in a human but not in Renilla. Because there are 64 possible combinations of the 4 DNA nucleotides in codon groups of 3, the genetic code is redundant for many of the 20 amino acids. Each of the different codons for a given amino acid encodes the incorporation of that amino acid into a polypeptide. However, within a given species there tends to be a preference for certain of the redundant codons to encode a given amino acid. The "codon preference" of *R. reniformis* is different from that of humans (this codon preference is usually based upon differences in the level of expression of the tRNAs containing the corresponding anticodon sequences). In order to obtain high expression of a non-human gene product in human cells, it is advantageous to change one or more non-preferred codons to a codon sequence that is preferred in human cells. Table 1 shows the preferred codons for human gene expression. A codon sequence is preferred for human expression if it occurs to the left of a given codon sequence in the table. Optimally, but not necessarily, less preferred codons in a non-human polynucleotide coding sequence are humanized by altering them to the codon most preferred for that amino acid in human gene expression. As used herein, a GFP is "humanized" if the amount of fluorescent polypeptide expressed in a human cell from a "humanized GFP" polynucleotide sequence is at least two-fold greater, on either a mass or a fluorescence intensity scale per cell, than the amount expressed from an equal amount or number of copies of, a non-humanized GFP polynucleotide.

Summary of Invention Paragraph - BSTX (97):

[0095] As used herein, "R. reniformis green fluorescent protein" or "R. reniformis GFP" refers to a polypeptide comprising the amino acid sequence of SEQ ID NO 2 or a fluorescent variant thereof. An *R. reniformis* GFP variant encompasses polypeptides of amino acid sequence SEQ ID NO: 2 that bear one or more mutations, including insertion or deletion of one or more amino acids, either at the N or C termini of the polypeptide or internal to the coding sequence. Variants of *R. reniformis* GFP retain the ability to emit light when excited by light within a given part of the spectrum, and may be excited by light of, or emit light in a portion of the spectrum that differs detectably from that which excites or which is emitted by wild-type *R. reniformis* GFP of amino acid sequence SEQ ID NO: 2. In addition to variants exhibiting different excitation or emission spectra, *R. reniformis* GFP variants include variants exhibiting increased fluorescence intensity relative to wild-type *R. reniformis* GFP. Preferably, a *R. reniformis* GFP, according to the invention, is encoded by a polynucleotide sequence comprising at least one humanized codon.

Summary of Invention Paragraph - BSTX (100):

[0098] As used herein, "identifying cells" refers to methods of identifying a GFP fluorescent cell within a population of cells. A "GFP-fluorescent cell", as used herein, refers to a cell that expresses both a R. reniformis luciferase fusion polypeptide gene and a humanized R. reniformis GFP fusion polypeptide gene in a manner effective to result in the production of the R. reniformis GFP fusion protein in an amount sufficient to allow subsequent identification of the cell by detecting BRET-induced green fluorescence from GFP in the cell. GFP-fluorescent cells may be identified by a variety of methods, including microscopy and fluorescence activated cell sorting (FACS).

Brief Description of Drawings Paragraph - DRTX (3):

[0104] FIG. 2 shows a the nucleotide sequence alignment between non-humanized (SEQ ID NO: 1) and humanized R. reniformis GFP (SEQ ID NO: 3). with the corresponding amino acid sequence depicted below the nucleotide sequence alignment (SEQ ID NO:2).

Detail Description Paragraph - DETX (3):

[0110] The invention provides for methods of detecting protein:protein interactions, methods of determining the location of a protein:protein interaction, and a method of screening for a candidate modulator that increases or decreases the amount of a protein:protein interaction, wherein these methods utilize a first fusion protein comprising an R. reniformis GFP protein fused to a first polypeptide encoded by a humanized nucleotide sequence and a second fusion protein comprising a R. reniformis luciferase protein fused to a second polypeptide.

Detail Description Paragraph - DETX (29):

[0136] c. Generation of Humanized GFP cDNA Sequences

Detail Description Paragraph - DETX (41):

[0148] cDNA sequences encoding R. reniformis luciferase (see FIG. 4) and cDNA sequences encoding R. reniformis GFP (see FIG. 2), according to the invention, are fused in frame to polynucleotide sequences encoding polypeptide domains of interest, via cloning methods well-known in the art (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., and Current Protocols in Molecular Biology, Ausubel, F. M. et al. (eds.) Greene Publishing Associates, (1989)). It is assumed that one of skill in the art can, given the polynucleotide sequences disclosed herein or those accessible on Genbank, readily construct genes comprising i) a polynucleotide sequence encoding R. reniformis Luciferase fused in frame with a sequence comprising one or more polypeptides or polypeptide domains of interest and ii) a humanized polynucleotide sequence encoding R. reniformis GFP fused in frame with a sequence comprising one or more polypeptides or polypeptide domains of interest. As used herein, the term "polypeptide of interest" or "domain of interest" refers to any polypeptide or polypeptide domain one wishes to fuse to either a R. reniformis luciferase or



R. reniformis **GFP** molecule of the invention. Again, the polynucleotide sequences encoding the polypeptide domains of interest are isolated by PCR or by isolation of a restriction enzyme digested DNA fragment according to recombinant DNA techniques well known to a person of the art. The fusion of R. reniformis **luciferase** or R. reniformis **GFP** polypeptide of the invention with a polypeptide of interest is made through linkage of the R. reniformis **luciferase** or **humanized R. reniformis GFP coding sequence** to either the N or C terminus of the fusion partner, according to methods well-known in the art. Mammalian expression vectors comprising humanized R. reniformis **GFP** (phrGFP-N1 and phrGFP-C, see FIG. 3) are commercially available and are described in Stratagene's online newsletter (B. Rogers et al., Strategies 13, 141-144 (2000)). The use of R. reniformis phrGFP-N1 and R. reniformis phrGFP-C vectors including detailed cloning procedures are available from Stratagene, La Jolla, Calif. 92037.

Detail Description Paragraph - DETX (107):

[0214] **GFP**-derived peptides used to induce specific antibodies preferably have an amino acid sequence consisting of at least five amino acids and more conveniently at least ten amino acids. It is advantageous for such peptides to be identical to a region of the natural R. reniformis **GFP** protein, and they may even contain the entire amino acid **sequence of wild-type or humanized R. reniformis GFP** (e.g., SEQ ID NO: 2).

Detail Description Paragraph - DETX (175):

[0282] Transgenic animals comprising a first fusion protein comprising a first polypeptide domain and a R. reniformis **GFP** (encoded by a **humanized polynucleotide sequence**) and a second fusion protein comprising a second polypeptide domain and a R. reniformis **luciferase** are prepared as described herein. A protein:protein interaction between the first and second polypeptide domains is detected in the transgenic animal by performing fluorescent microscopy on the various organs of the transgenic animal or on tissue sections prepared from the transgenic animal. Alternatively, a cell type of interest is isolated from the transgenic animal and analyzed for fluorescent emission in an instrument capable of detecting BRET-induced fluorescence or by FACS.

Detail Description Paragraph - DETX (177):

[0284] The subcellular location of a protein:protein interaction is determined by performing fluorescence microscopy on cells transfected with a vector encoding a first fusion protein comprising a first polypeptide domain and a R. reniformis **GFP** (encoded by a **humanized polynucleotide sequence**) and a second fusion protein comprising a second polypeptide domain and a R. reniformis **luciferase**, as described herein.

Detail Description Paragraph - DETX (179):

[0286] The components of the BRET system (i.e. a first fusion protein comprising a first polypeptide domain and a R. reniformis **GFP** (encoded by a **humanized polynucleotide sequence**) and a second fusion protein comprising a second polypeptide domain and a R. reniformis) **Luciferase** can be produced using

molecular biology techniques, as described herein, or isolated from natural sources. After purification, they can be used in non-cell based in vitro assays (as described in Example 1).

Detail Description Paragraph - DETX (185):

[0292] The components of the BRET system (i.e. a first fusion protein comprising a first polypeptide domain and a R. reniformis **GFP** (encoded by a **humanized polynucleotide sequence**) and a second fusion protein comprising a second polypeptide domain and a R. reniformis) **Luciferase** can be produced using molecular biology techniques, as described herein, or isolated from natural sources. After purification, they can be used in non-cell based in vitro assays in the presence and absence of a candidate modulator of the invention.

Detail Description Paragraph - DETX (195):

[0300] A first fusion protein comprising a nucleotide **sequence encoding the EGF receptor fused in frame to a humanized nucleotide sequence** encoding a R. reniformis **GFP** polypeptide and a second fusion protein comprising a nucleotide sequence encoding EGF fused in frame to a nucleotide sequence encoding a R. reniformis **luciferase** polypeptide are produced by any of the methods described herein.

Detail Description Paragraph - DETX (200):

[0304] A cell line useful according to the invention is transected with a first vector encoding a fusion protein comprising a nucleotide **sequence encoding the EGF receptor fused in frame to a humanized nucleotide sequence** encoding a R. reniformis **GFP** polypeptide. The first vector also comprises a neomycin resistance gene. The cells are also transfected with a second vector encoding a fusion protein comprising a nucleotide sequence encoding EGF fused in frame to a nucleotide sequence encoding a R. reniformis **luciferase** polypeptide. The second vector also comprises a hygromycin resistance gene.

Detail Description Paragraph - DETX (206):

[0309] A cell line useful according to the invention is transected with a first vector encoding a fusion protein comprising a nucleotide **sequence encoding the EGF receptor fused in frame to a humanized nucleotide sequence** encoding a R. reniformis **GFP** polypeptide. The first vector also comprises a neomycin resistance gene. The cells are also transfected with a second vector encoding a fusion protein comprising a nucleotide sequence encoding EGF fused in frame to a nucleotide sequence encoding a R. reniformis **luciferase** polypeptide. The second vector also comprises a hygromycin resistance gene.

Claims Text - CLTX (2):

2. The composition of claim 1, wherein the polynucleotide **sequence of R. reniformis GFP contains at least one codon which is humanized.**

Claims Text - CLTX (11):

11. The composition of claim 10, wherein the polynucleotide sequence encoding R. reniformis GFP polypeptide is humanized.

Claims Text - CLTX (21):

21. The composition of claim 20, wherein the polynucleotide sequence of R. reniformis GFP contains at least one codon which is humanized.

Claims Text - CLTX (29):

29. The composition of claim 28, wherein the polynucleotide sequence of R. reniformis GFP contains at least one codon which is humanized.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030154502 A1

TITLE: Universal markers of transgenesis

PUBLICATION-DATE: August 14, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 360222

DATE FILED: February 7, 2003

RELATED-US-APPL-DATA:

child 10360222 A1 20030207

parent continuation-of 09373129 19990812 US GRANTED

parent-patent 6518481 US

US-CL-CURRENT: 800/8, 800/14 , 800/19 , 800/20 , 800/21

ABSTRACT:

The invention relates to methods, cells and nucleic acids for making transgenic animals. The methods generally comprise introducing into a genome of an animal a genetic construct comprising a transcriptional regulatory element operably linked to a heterologous marker gene encoding a marker, wherein the element drives expression of the marker across genera transgenic in the construct sufficient to visually detect the marker in photoreceptive cells or organs, and selecting for transgenesis by visually detecting the marker in a photoreceptive cell or organ of the animal.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority under 35USC120 to U.S. Ser. No. 09/373,129, having the same title and inventors, filed on Aug. 12, 1999, now U.S. Pat. No. 6,518,481, which is incorporated herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX (13):

[0011] To drive marker expression in a series of diverged organisms requires a promoter which is active in a wide range of species. Furthermore, to avoid problems with low expression and the interference of autofluorescence, a regional specific promoter is preferable over a constitutively active one. A wide variety of **regulatory** elements may be employed, so long as they meet the requisite functional limitations. These may be natural promoter elements, naturally driving gene expression in photoreceptive cells or organs, elements derived from such natural promoter elements by **mutational** selection or consensus **sequences**, **synthetic** elements derived by iterative selection process, e.g. SELEX procedures, etc. In a particular embodiment, the element comprises a binding site selected from a Pax-6, a Pax-6 like binding site such as a twin-of-eyeless (TOY) binding site, a Glass binding site, etc. In more particular embodiments, the element comprises a Pax-6 Paired Domain or Homeodomain binding site, more particularly a P3 site, wherein the P3 site comprises the sequence: TAATYNRATTA (SEQ ID NO:01), wherein Y=C or T; R=G or A; N=any nucleotide (Wilson et al., 1993, Genes Dev 7, 2120-34; Czemy and Busslinger, 1995, Mol Cell Biol 15, 2858-71). Tables 1-6 provide other exemplary transcriptional **regulatory** element binding sites functional in the subject methods. Pax-6 binding sites are of particular interest due to the evolutionary conserved role Pax-6-homologs play in eye development across different phyla (Callaerts et al., 1997, Annu Rev Neurosci 20, 483-532).

Summary of Invention Paragraph - BSTX (23):

[0021] Fluorescent proteins may comprise naturally occurring, engineered (i.e., analogs) and/or **synthetic sequences**. For example, many cnidarians use natural green fluorescent proteins ("GFPs") as energy-transfer acceptors in bioluminescence. Natural GFPs have been isolated from numerous animals, including the Pacific Northwest jellyfish, *Aequorea victoria*, the sea pansy, *Renilla reniformis*, and *Phialidium gregarium*; Ward et al., Photochem. Photobiol., 35:803-808 (1982); Levine et al., Comp. Biochem. Physiol., 72B:77-85 (1982). In addition, a variety of *Aequorea*-related fluorescent proteins having useful excitation and emission spectra have been engineered by modifying the amino acid sequence of a naturally occurring **GFP** from *Aequorea victoria* (Prasher et al., Gene, 111:229-233 (1992); Heim et al., Proc. Natl. Acad. Sci., USA, 91:12501-04 (1994). Particularly useful are GFPs from or which derive from the jellyfish *A. victoria* (see e.g. U.S. Pat. No. 5,491,084 for applicable such GFPs) and include variants offering a variety of different excitation and emission wavelengths; see e.g. Heim and Tsein, 1996, Current Biology 6, 178-182. Exemplary amino acid variants include F64L, S65T, Y66W, N146I, M153T, V163A and N212K, and combinations thereof. For example, CFP is the **GFP** of *Aequorea victoria* with the following additional **mutations**: F64L, S65T, Y66W, N146I, M153T, V163A, N212K (Miyawaki et al., 1997, Nature 388:882-7), and YFP is the **GFP** of *A. victoria* with the following additional **mutations**: S65G, V68L, S72A, T203Y (Cubitt et al., 1999, Methods Cell Biol 58, 19-30). Accordingly, in preferred embodiments, the marker is a *Aequorea* or *Aequorea*-related fluorescent protein, see U.S. Pat. No. 5,912,137 for applicable sequence, scope, definitions and examples.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030150004 A1

TITLE: Screening systems and methods for identifying  
modulators of xenobiotic metabolism

PUBLICATION-DATE: August 7, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 268822

DATE FILED: October 10, 2002

RELATED-US-APPL-DATA:

child 10268822 A1 20021010

parent continuation-in-part-of PCT/US01/29672 20010921 US PENDING

child 10268822 A1 20021010

parent continuation-in-part-of 10219590 20020815 US PENDING

US-CL-CURRENT: 800/18

ABSTRACT:

The present invention provides mice having reduced CAR receptor activity and mice expressing a human CAR receptor. These mice are useful in screening methods to identify CAR ligands, including compounds that modulate CAR receptor activity, compounds likely to have CAR-mediated toxicity, and analogs of these compounds with less potential toxicity.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of International Application PCT/US01/29672, filed Sep. 21, 2001 and published in English, and a continuation-in-part of U.S. utility application 10/219,590, filed Aug. 15, 2002, both of which claim priority to U.S. utility application Ser. No. 09/666,250, filed Sep. 21, 2000.

----- KWIC -----

Brief Description of Drawings Paragraph - DRTX (16):

[0070] FIG. 10 is a picture of a Northern blot illustrating induction of the CAR target gene CYP2B10 by PB, but not by TCPOBOP, in humanized CAR mice lacking a functional murine CAR gene. Animals with the indicated genotypes (with, "+," or without, "-", the line 6210 hCAR transgene, and heterozygous, "-/+", or homozygous, "-/-," for the murine CAR gene mutation) were treated with PB or TCPOBOP for 24 hours. Total RNA was prepared from the livers of the individual mice, and CYP2B10 mRNA expression was assessed by Northern blotting.

Detail Description Paragraph - DETX (29):

[0110] Various promoters known in the art may be used for directing expression of the human CAR receptor. Examples of such promoters for expression of transgenes include, but are not limited to the zinc-inducible metallothionein promoter, the DEX-inducible tyrosine aminotransferase (TAT) promoter, the rifampicin inducible CYP3A4 promoter, the albumin promoter (Xie et al., Nature 406:435-439, 2000), the fatty acid binding protein (FABP), transthyretin (TTR) (Ye et al., Mol Cell Biol. 19:8570-8580, 1999), or CAR promoter. The FABP promoter is expressed in the liver and small intestine. The TTR promoter is also well described and widely used promoter to achieve liver-specific expression of transgenes. Other regulatory sequences may be used to enhance expression of the human CAR receptor in a humanized CAR mouse.

Detail Description Paragraph - DETX (40):

[0121] Moreover, as alternatives to assays involving endogenous murine CAR target genes, assays may be conducted to measure appropriate reporter transgenes inserted by any standard technique (for example, those techniques described above) into wild-type mice, CAR -/- mice, humanized CAR mice, mice lacking the gene encoding the receptor related to CAR (known as SXR, PXR or by other names (Kliwer et al., Cell 92:73-82, 1998; Blumberg et al., Genes Dev. 12:3195-3205, 1998)), or any other appropriate strain. These reporter transgenes consist of a CAR responsive promoter operably-linked to an easily measured reporter gene. Examples of appropriate promoters include native CYP promoters such as the CYP2B10 promoter containing the previously described phenobarbital response element (Honkakoski et al., supra), the CYP2B6 promoter, the CYP3A11 promoter, the CYP3A4 promoter, or synthetic promoter constructs in which DNA binding sites for CAR/RXR heterodimers are linked to functional basal promoters (Tzameli, et al., supra). Examples of appropriate reporter genes include, without limitation, human growth hormone, secreted alkaline phosphatase, luciferase, green fluorescent protein, chloramphenicol acetyl transferase, CYP2B6, CYP3A11. and any other reporter gene (see, for example, Ausubel et al. (Chapter 9), supra). The assays for CAR target genes involve standard procedures (see, for example, Ausubel et al. (Chapter 9), supra) and may be based on appropriate samples from the mice, such as liver or serum samples. Alternatively, hepatocytes or other appropriate cell types may be harvested from the animals and propagated. Compounds may be administered to these cells to determine whether the compounds effect a change in expression of CAR target genes or reporter transgenes.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030149254 A1

TITLE: Methods and compositions comprising Renilla GFP

PUBLICATION-DATE: August 7, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 133973

DATE FILED: April 24, 2002

RELATED-US-APPL-DATA:

child 10133973 A1 20020424

parent continuation-of 09710058 20001110 US PENDING

non-provisional-of-provisional 60290287 20010510 US

non-provisional-of-provisional 60164592 19991110 US

US-CL-CURRENT: 536/23.1, 435/320.1, 435/325, 435/6, 435/69.7, 530/350

ABSTRACT:

The invention relates to methods and compositions utilizing Renilla green fluorescent proteins (rGFP), and Ptilosarcus green fluorescent proteins (pGFP). In particular, the invention relates to the use of Renilla GFP or Ptilosarcus GFP proteins as reporters for cell assays, particularly intracellular assays, including methods of screening libraries using rGFP or pGFP.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of the filing date of application U.S. Serial No. 60/290,287 filed May 10, 2001 and of application U.S. Ser. No. 09/710,058, filed Nov. 10, 2000.

----- KWIC -----

Detail Description Paragraph - DETX (56):

[0078] The codon optimized GFPs are made in accordance with methods well



known in the art. When the substitutions or replacements are not extensive, oligonucleotide directed **mutagenesis** or other localized **mutagenesis** techniques, such as replacing fragments of the gene with fragments containing the preferred codons, are used to optimize the codons. If codon optimization is extensive, the **GFP gene may be a synthetic gene** generated from overlapping oligonucleotides (Jayaraman, K. et al. (1991) Proc. Natl. Acad. Sci. USA 88: 4084-8; Stemmer, W. P. et al. (1995) Gene 164: 49-53). The oligonucleotides may or may not be ligated together during the process for generating the **synthetic gene**. In this regard, use of polymerase chain reaction of the hybridized overlapping oligonucleotides allows facile generation of these **synthetic genes**.

Detail Description Paragraph - DETX (130):

[0152] Promoter sequences are either constitutive or inducible promoters. By "promoter" herein is meant nucleic acid sequences capable of initiating transcription of the fusion nucleic acid or portions thereof. Promoters may be constitutive wherein the transcription level is constant and unaffected by modulators of promoter activity. Promoter may be inducible in that promoter activity is capable of being increased or decreased, for example as measured by the presence or quantitation of transcripts or translation products (see Walter, W. et al. (1996) J. Mol. Med. 74: 379-92). Promoters may also be cell specific wherein the promoter is active only in particular cell types. Thus, promoter as defined herein includes sequences required for initiating and **regulating** the transcription level and transcription in specific cell types. Furthermore, the promoters may be either naturally occurring promoters, hybrid promoters which combine elements of more than one promoter, or **synthetic promoters based on consensus sequence** of known promoters.

Detail Description Paragraph - DETX (353):

[0372] pR and pP are retroviral expression vectors comprising Renilla muelleri and Ptilosarcus gumei GFPs (containing 9 and 11 non-optimized codons, respectively, to introduce restriction sites). Each has a Kozak consensus start and backbone vector sequence identical to that of pCGFP and pEF. These vectors were made by annealing and ligating 20 **synthetic oligonucleotides (10 forward, 10 reverse for each GFP gene)** creating a dsDNA fragment for each sequence shown in Table 1. These fragments were PCR amplified with respective primers:

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030148340 A1

TITLE: Method and kits for preparing multicomponent nucleic acid constructs

PUBLICATION-DATE: August 7, 2003

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

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child 09220398 19981224 US

parent continuation-in-part-of 08877034 19970616 US GRANTED

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non-provisional-of-provisional 60019869 19960617 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US97/10523	1997WO-PCT/US97/10523	June 16, 1997

US-CL-CURRENT: 435/6, 435/91.2

ABSTRACT:

The invention provides a highly efficient, rapid, and cost effective method of linking nucleic acid components in a predetermined order to produce a nucleic acid multicomponent construct. The invention further provides nucleic acid components, each nucleic acid component comprising a double stranded nucleic acid molecule having at least one single stranded 5' or 3' terminal sequence, the terminal sequence having sufficient complementarity to either a terminal sequence in a separate nucleic acid component or to a sequence in a linking

nucleic acid molecule so as to allow for specific annealing of complementary sequences and linkage of the components in a predetermined order. Kits containing reagents required to practice the method of the invention are also provided.

## RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. Ser. No. 08/877,034, filed Jun. 16, 1997, which claims the benefit of a previously filed Provisional Application No. 60/019,869 filed Jun. 17, 1996, the specifications of which are hereby incorporated by reference.

----- KWIC -----

### Detail Description Paragraph - DETX (106):

[0134] As used herein, the terms "exon" and "exonic sequence" denotes nucleic acid sequences, or exon "modules", that can, for instance, encode portions of proteins or polypeptide chains, such as corresponding to naturally occurring exon sequences or naturally occurring exon sequences which have been mutated (e.g. point mutations, truncations, fusions), as well as nucleic acid sequences from "synthetic exons" including sequences of purely random construction. However, the term "exon", as used in the present invention, is not limited to protein-encoding sequences, and may comprises nucleic acid sequences of other function, including nucleic acids of "intronic origin" which give rise to, for example, ribozymes or other nucleic acid structure having some defined chemical function.

### Detail Description Paragraph - DETX (123):

[0151] As suggested above, the inducible promoters of the present invention include those which are not naturally occurring promoters but rather synthetically derived inducible promoter systems which may make use of prokaryotic transcriptional repressor proteins. The advantage of using prokaryotic repressor proteins in the invention is their specificity to a corresponding bacterial operator binding site, which can be incorporated into the synthetic inducible promoter system. These prokaryotic repressor proteins have no natural eukaryotic gene targets and affect only the effector of suppression gene which is put under the transcriptional control of the inducible synthetic promoter. This system thereby avoids undesirable side-effects resulting from unintentional alteration of the expression of nontargeted eucaryotic genes when the inducible promoter is induced. A preferred example of this type of inducible promoter system is the tetracycline-regulated inducible promoter system. Various useful versions of this promoter system have been described (see Shockett and Schatz (1996) Proc. Natl. Acad. Sci. USA 93: 5173-76 for review). As suggested above, these tetracycline-regulated systems generally make use of a strong eucaryotic promoter, such as human cytomegalovirus (CMV) immediate early (IE) promoter/enhancer and a tet resistance operator (tetO) which is bound by the tet repressor protein. In a preferred embodiment, the system involves a modified version of the tet repressor protein called a reverse transactivator (rtTA, or rtTA-nls, which contains a nuclear localization signal) which binds

tetO sequences only in the presence of the tet derivatives doxycycline or anhydrotetracycline. Using this system, a synthetic human CMV/IE-tetO-promoter driven construct could be induced by 3 orders of magnitude in 20 hrs by the addition of the tet derivatives (see Gossen et al. (1995) Science 268: 1766-9). Thus this system can be used to make the effector of suppression genes of the present invention inducible in response to the delivery of tetracycline derivatives to the targeted eucaryotic cell. Alternatively, a tet repressor fused to a transcriptional activation domain of VP16 (tTA) can be used to drive expression of the inducible promoter of the present invention. In this instance, transcriptional activation of a synthetic human CMV/IE-tetO-promoter driven construct is achieved by the removal of tetracycline since the tTA activator only binds to the tetO in the absence of tet (see Gossen and Bujard (1992) Proc. Natl. Acad. Sci. USA 89: 5547-51). Other synthetic inducible promoter systems are also available for use in the present invention. For example, a lac repressor-VP16 fusion which exhibits a "reverse" DNA binding phenotype (i.e., analogous to rTA described above, it only binds the lacO operator sequence in the presence of the inducer IPTG) (see Lambowitz and Belfort (1993) Annu Rev Biochem 62: 587-622). This particular synthetic inducible promoter is approximately 1000-fold inducible in the presence of IPTG. Since neither the tet repressor gene nor the lac repressor gene occurs naturally in a eukaryotic cell, systems involving synthetic inducible promoter constructs such as these rely on the further delivery of an expressible copy of the appropriate prokaryotic repressor gene. Suitable expression cassettes for this purpose are readily available for heterologous expression in many different eukaryotic cells including various yeast species and mammalian cells.

Detail Description Paragraph - DETX (125):

[0153] Yet another vector element of the present invention is fusion polypeptide-encoding element which can be fused to the coding region of any insert gene of interest. For example, in certain applications it is useful to be able to mark a particular gene product with a tag so that the localization and function of the gene product can be easily monitored. A particularly preferred version of a molecular tag fusion polypeptide element is the green fluorescent protein or GFP (see e.g. Misteli and Spector (1997) Nature Biotechnology 15: 961-4; and Gerdes and Kaether (1996) FEBS Lett 389: 44-7 for review). This fusion tag polypeptide emits green (approximately 510 nm wavelength) light upon excitation by a particular wavelength of incident light (approximately 400 to 480 nm, depending upon the form of GFP). Various versions of GFP coding sequences, including those whose codon usage has been humanized and those whose emission spectra have been "red-shifted," are commercially available and can be readily adapted to GEOS methodology. Applications of GFP include in situ localization of a linked gene of interest, as well as facile monitoring of expression and tropism in various cell mediated expression studies.

Detail Description Paragraph - DETX (136):

[0164] A vector may be assembled from multiple individual nucleic acid components, including, without limitation, nucleic acid components which incorporate one or more of the following: (a) origin of replication (bacterial, viral, phage, yeast, mammalian, eukaryotic), (b) selectable markers (antibiotic resistance, drug resistance, mutagenic resistance), (c) promoters (phage,

bacterial, yeast, eukaryotic, mammalian), (d) regulatory elements or genes (repressors, enhancers), (e) structural genes, (f) fragments of structural genes, (g) translational elements (Shine-Delgarno element, Kozak sequence), (h) terminators of transcription, (i) regulators of mRNA stability (degradation signals, translational regulators), (j) protein encoded elements specifying cellular location (leader sequence, KDEL, CAAX box, nuclear targeting elements), (k) recombination elements (Lox-CRE, M13 ori), (l) mutagenized genes, (m) protein domain encoded regions, (n) synthetic multiple cloning sites, (o) unique restriction enzyme or DNA cleavage sites, (p) site for covalent or non covalent attachment of a biological or chemical molecule (see "Handle").

Claims Text - CLTX (15):

15. The method of claim 1, wherein the nucleic acid component encodes a biological functionality selected from the group consisting of origin of replication, selectable marker, transcriptional regulatory element, structural gene or fragment thereof, transcription termination signal, translational regulatory sequence, regulators of mRNA stability, cellular localization signal, recombination elements, mutagenized genes, protein domain encoded regions, synthetic multiple cloning sites, unique restriction enzyme or DNA cleavage sites, and site for covalent or non covalent attachment of a biological or chemical molecule."

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030129170 A1

TITLE: Human tyrosine hydroxylase promoter and uses thereof

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

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DATE FILED: August 9, 2002

RELATED-US-APPL-DATA:

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parent continuation-in-part-of 09942325 20010829 US PENDING

non-provisional-of-provisional 60228931 20000830 US

US-CL-CURRENT: 424/93.21, 435/191, 435/320.1, 435/368, 435/69.1  
, 435/7.21, 536/23.2

ABSTRACT:

The present invention provides an isolated, purified and characterized human tyrosine hydroxylase (hTH) promoter nucleic acid sequence. The invention further provides a method of selecting TH positive (TH+) cells by preparing a construct comprising a hTH promoter operably linked to a heterologous nucleic acid sequence, for example, green fluorescent protein encoding sequence, and transfecting cells, particularly stem cells, with the construct. The invention also provides a hTH promoter, useful in gene therapeutic applications in driving therapeutic genes or other nucleic acid sequences operably linked to the hTH promoter. Additionally, the invention provides cell lines and transgenic animals expressing a transgene comprising the hTH promoter operably linked to a heterologous sequence, which cell lines and transgenic animals are useful for isolating TH+ cells for transplantation or for screening of therapeutic agents that affect TH+ function. Methods of producing cell lines and transgenic animals also provided.

RELATED APPLICATIONS

[0001] This is a continuation-in-part application of the U.S. patent application, Ser. No. 09/942,325, filed Aug. 29, 2001, which claims priority

----- KWIC -----

Brief Description of Drawings Paragraph - DRTX (9):

[0036] FIGS. 8A-8I shows a schematic of a minipromoter. FIG. 8A marks the nucleotide positions with respect to the start of transcription (Exon I in B). FIG. 8B shows the 5' end of the human TH gene is represented, including 11 kb of promoter and the first Exons. BBE's I-IV are recognition sites for the transcription factor bicoid, associated with regulation of the TH gene by Pitx3. FIG. 8C shows the repeat sequences within the human TH promoter including a previously described tetranucleotide (TTCA) repeat in the first intron. FIG. 8D shows the locations of select potential response elements. These include the early response factors EGR and AP-1-4; a single cyclic-AMP response element (CRE), a Gli site which may mediate Shh action, and two 70% matches to the neuron restrictive silencer element, NRSE. FIG. 8E shows a Human TH promoter. Solid black squares denote Conserved Regions I-V. FIG. 8F is a 12,007 bp transgene used to generate hTH-11 kb-EGFP transgenic mice. FIG. 8G shows a "minipromoter" and reporter construct. The 12,007 construct has been cleaved at a unique internal restriction site, and a synthetic gene sequence (\*) inserted. The bars in this region reflect the numerous sequence elements from B,D, and E above assembled in a 500-1000 bp synthetic sequence. These include EGR, AP-1-4, BBE's I through III, the 5' NRSE, and conserved regions I-IV. To encompass all the potential response elements illustrated in B through E (above) requires less than 300 bp of total sequence. To be conservative, and to allow for space between elements so that transcription factors are not subject to steric hinderance, we allot up to 1 kb for this synthetic region. This is quite manageable for chemical/enzymatic gene synthesis. The rest of the hTH promoter is in white, the crosshatch represents the reporter (GFP), while the SV40 polyadenylation sequence is in black. FIG. 8H is a diagram of a typical 3.sup.rd generation lentivirus vector (Ailles,L. E. and Naldini,L., HIV-1-Derived Lentiviral Vectors. In: Trono,D. (Ed.), Lentiviral Vectors, Springer-Verlag, Berlin, Heidelberg, New York, 2002, pp. 31-52) drawn roughly to scale. The "cargo" includes a generic promoter ("prom") and reporter ("TRANSGENE"). FIG. 8I shows a design of a lentiviral vector incorporating an hTH minipromoter driving expression of GFP. The entire vector/transgene construct is about 11-12 kb, well within range of the 8-14 kb packaging capacity of the virus.

Detail Description Paragraph - DETX (95):

[0132] Strategy used for modification of native human tyrosine hydroxylase (hTH) promoter. Only the "top" strand of a portion of the sequence is depicted for ease of alignment. Numbers at top characterize the proximal portion of the hTH transcript that begins with +1. Immediately below is the native hTH sequence with transcribed sequence in CAPS, and translated sequence. Below this is the reverse complement of synthetic oligonucleotide MAKIL 124 used for PCR-mediated mutation. The mutating sequence is in BOLD ITALICS. Note that the mutation creates a Bgl II site (AGATCT) which after digestion (\*) yields the final sequence. This unique synthetic Bgl II enables ligation to reporter

sequence bearing a compatible cohesive end (e.g. Bgl II, BamHI, Bcl etc.) Note in particular that this mutation leaves unchanged the native sequence of the 5' untranslated region through +14.



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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030124708 A1

TITLE: Modification of virus tropism and host range by viral  
genome shuffling

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 310741

DATE FILED: December 4, 2002

RELATED-US-APPL-DATA:

child 10310741 A1 20021204

parent continuation-of 09183037 19981030 US PENDING

child 09183037 19981030 US

parent continuation-in-part-of 08962236 19971031 US ABANDONED

US-CL-CURRENT: 435/235.1, 435/239 , 435/346 , 435/456 , 536/23.72

ABSTRACT:

The invention relates to a method and compositions for modifying a phenotype of a virus, such as viral tropism and host range, by iterative sequence recombination of variant viruses and selection of improved variants.

CROSS REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of Ser. No. 08/962,197 filed Aug. 31, 1997. The present application claims benefit of the Ser. No. 08/962,197 application, which is incorporated herein by reference in its entirety for all purposes.

----- KWIC -----

Detail Description Paragraph - DETX (93):

[0130] Transgenes and expression vectors can be constructed by any suitable method known in the art. It is often desirable to generate coding sequences for CD4, CCR5, CXCR4, and other human accessory proteins that aid viral infectivity by either PCR or RT-PCR amplification from a suitable human cell type (erg., a T lymphocyte population) or by ligating or amplifying a set of overlapping synthetic oligonucleotides; publicly available sequence databases and the literature can be used to select the polynucleotide sequence(s) to encode the specific protein desired, including any mutations, consensus sequence, or mutation kernel desired by the practitioner. The coding sequence(s) are operably linked to a transcriptional regulatory sequence (e.g., T cell lineage-specific promoter/enhancer) and, if desired, an origin of replication (e.g., EBV ori) for episomal replication, or one or more flanking sequences having substantial sequence identity to a host chromosomal sequence to provide for homologous recombination and targeted integration of the transgene. In an embodiment, a transgene comprises a human CD4 minigene or a substantially complete human CD4 gene. Similar transgenes comprise a CCR5 and/or CXCR4 minigene or substantially complete gene. The transgenes can use the native gene transcriptional regulatory sequences, or can employ an operably linked heterologous transcriptional regulatory sequence (e.g., a mouse CD4 promoter/enhancer, a CMV promoter/enhancer, a human T cell receptor gene promoter/enhancer, and the like). Often the transgene(s) and expression vector(s) will further comprise a reporter gene or a selectable marker gene (e.g., tk, neo) in a selection cassette to facilitate identification and enrichment of cells having the construct in functional form.

Detail Description Paragraph - DETX (319):

[0348] In one embodiment, the following steps are used to evolve HIV for replication in non-human cells. First, cloning vectors and protocols for shuffling infectious molecular clones in two non-infectious pieces are established. Second, methods for efficiently making large (>10<sup>6</sup> complexity) libraries of infectious molecules from shuffled fragments of HIV-1 are established. Third, libraries of HIV-1 recombinants are produced using in vivo recombination pathways. Fourth, synthetic HIV-1 tat genes representing all clades are bred into infectious virus and make large libraries of infectious molecular clones, based on the techniques established in the first and second step. Fifth, CD4<sup>+</sup> CCR5<sup>+</sup> mouse cells are infected with these libraries and selected for mutants which can replicate and be passaged in tissue culture. Eight consensus tat genes representing all eight HIV-1 clades were synthesized. Sixth, mouse and human reporter cells containing constructs with GFP under the control of an HIV-1 LTR were constructed. Seventh, the transcriptional activation activities of the synthetic tat genes in the reporter cells are validated. Ninth, libraries of shuffled tat genes are constructed in an MLV retroviral vector and introduce them into the reporter cells. Tenth, methods to FACS select libraries for cells with tat induced GFP are established, and the tat genes recovered by PCR, shuffled, and reconstruct new libraries of shuffled genes. Eleventh, evolved tat genes are bred into the context of infectious molecular clones and select for growth on mouse cells. For Macaques, an immortalized macaque cell line is constructed expressing macaque CD4 and CCR5, which are known to support efficient entry of HIV into macaque cells. This cell line is used to select in tissue culture for replication of HIV in macaque cells.

PGPUB-DOCUMENT-NUMBER: 20030092043

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030092043 A1

TITLE: Melanoma differentiation associated gene - 5 and  
promoter and uses thereof

PUBLICATION-DATE: May 15, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 228897

DATE FILED: August 26, 2002

RELATED-US-APPL-DATA:

child 10228897 A1 20020826

parent continuation-of PCT/US01/06960 20010228 US PENDING

child PCT/US01/06960 20010228 US

parent continuation-of 09515363 20000229 US PENDING

US-CL-CURRENT: 435/6, 435/184 , 435/320.1 , 435/325 , 435/69.1 , 435/7.23  
, 536/23.2

ABSTRACT:

The invention provides for an isolated nucleic acid encoding Mda-5 (melanoma differentiation associated gene-5) and an isolated Mda-5 polypeptide. The invention further provides a vector comprising the nucleic acid encoding Mda-5, as well as a host cell comprising the vector. The invention provides an antibody which specifically binds to an Mda-5 polypeptide. The invention further provides a method for determining whether a compound is an inducer of Mda-5 gene expression and assays to determine whether a compound modifies the enzymatic activity of the Mda-5 polypeptide.

[0001] This application is a continuation of U.S. Ser. No. 09/515,363, filed Feb. 29, 2000, the contents of which are hereby incorporated by reference.

----- KWIC -----

Detail Description Paragraph - DETX (82):

[0102] A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. For example, a sequence encoding a protein other than an Mda-5 is considered a heterologous sequence when linked to an Mda-5 promoter. Similarly, a sequence encoding an Mda gene (i.e., Mda-6, Mda-7) will be considered heterologous when linked to an Mda gene promoter with which it is not normally associated. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Likewise, a chimeric sequence, comprising a heterologous structural gene and a gene encoding an Mda or a portion of an Mda, linked to an Mda promoter, whether derived from the same or a different Mda gene, will be considered heterologous since such chimeric constructs are not normally found in nature. Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

Detail Description Paragraph - DETX (187):

[0205] The modified mda-5 reporter construct was transfected into parallel sets of HO-1 cells that were treated or not with IFN-.beta.. Quantitation of luciferase activity indicated that this modified reporter containing a partially deleted Exon 1 and around 6 kb of upstream sequences, showed .about.10 fold higher luciferase activity in cells that had been treated with IFN-.beta. compared to untreated controls. This level of induction was comparable to that seen with the endogenous gene in Northern blot analyses. It therefore appeared that the cloned regulatory genomic DNA sequence in the reporter construct contained the elements required for the regulation of the mda-5 gene. It was however, necessary to confirm that the cloned sequences contained all the regulatory elements involved in the transcriptional control of the endogenous cellular gene. Since HO-1 melanoma cells show a very low and variable transfection efficiency it initially proved very difficult to determine the activity of the transiently transfected reporter in a consistent way for variables such as kinetics of induction, optimal concentration of inducer and the identification of other potential activators. To circumvent this technical problem the mda-5 promoter construct was stably integrated into genomic DNA of HO-1 cells by co-transfection with a puromycin resistance plasmid and selection to isolate a clonal population of stable integrants. This selection procedure resulted in the production of several clones of which 48 were randomly picked for further analysis. Screening of these stable promoter clones by treatment with IFN-.beta. indicated that an entire range from completely inactive to highly active, as measured by luciferase activity, had been obtained (FIG. 11). Some of the clonal isolates showed induction levels similar to the endogenous gene (around 10 fold), while others displayed much higher induction (around 100-fold). It is a likely possibility that the clones showing higher levels of induction contain multiple copies of integrated plasmid that due to an additive effect show higher levels of activity. Two individual clones (#20 and #40) were selected for further analyses to determine if activation kinetics and overall responsiveness to inducer, as a measure to ascertain the completeness of the isolated promoter sequence, mimicked that previously observed for the endogenous gene. In the initial screen (FIG. 11)

the clone designated #20 showed a very low basal activity that on induction was >1000 fold. It was therefore not included in the plot to permit the scale to represent the other clones (ranging from 0-150 fold induction) accurately. This clone on subsequent analysis displayed a very low basal activity but a much lesser final fold activation (FIG. 12) than seen in the initial screen, but has maintained this property over several subsequent culture passages. To determine the induction kinetics of the promoter construct following treatment with IFN-.beta., a fixed number of cells (106/6 cm culture dish) was treated with inducer and assayed for **luciferase** activity compared with a parallel uninduced control sample, at various time-points following treatment (FIG. 12). Irrespective of the final fold-induction of **luciferase** levels, which varied in an individual clone, the overall pattern of induction kinetics was almost identical and similar to that of the endogenous gene as determined by Northern blotting. Similarly, assays were performed to determine the range of sensitivity of detection of exogenously added Interferon levels as determined by a **luciferase** read out (FIG. 13). The results of this assay closely paralleled that observed for the endogenous gene with measurable levels of 0.2 U of IFN-.beta. being detectable. The promoter clone isolates were also used to determine responsiveness to different forms of IFNs including human IFN-.alpha., .beta., and .gamma. synthetic double stranded RNA (polyIC:IC; Amersham) and TNF-.alpha. (FIG. 14A) using transient transfection assays with the reporter construct, in HO-1 melanoma cells. In addition, Clone #40 stable HO-1 cells were treated with human IFN-.alpha.A, -.alpha.b2, -.alpha.C, .alpha.D, .alpha.F, -.alpha.G, .alpha.H, -.alpha.I, -.alpha.J, -.alpha.A/D, PBL 1001, Bovine Tau, .OMEGA. and Human IFN-.beta. (FIG. 14B). Differential levels of responsiveness were seen dependent on the type of compound used, in general the Mda-5 promoter construct was most responsive to INF-.beta. relative to other IFNs, comparable to the results obtained in Northern analyses with the endogenous cellular gene. As seen above, Mda-5 **gene induction also occurs upon treatment of cells with synthetic** double stranded RNA (poly IC:IC). Studies identical to those described for IFN-.beta. were performed using double stranded RNA as an inducer with the stable HO-1 promoter clones. These experiments generated results that were similar to endogenous gene induction for parameters including time and level of induction (FIG. 15).

#### Detail Description Paragraph - DETX (197):

[0215] Taken together, the distinctive features of the MDA-5 protein suggest that this molecule represents a member of a new family of RNA helicases. If this is the case, mda-5 may participate in degradation, translation or inhibition of translation of pro or anti-apoptotic RNA molecules through its RNA helicase domain. Alternatively, mda-5 might be a signal transducer between IFN signals and the apoptotic machinery to prepare the cell for viral invasion and dsRNA accumulation. Localization of **GFP**-mda-5 fusion protein in the cytoplasm is not contradictory to this hypothesis. The reporter isolate comprising the mda-5 promoter sequences driving the **luciferase** cDNA, based on comparison of the quantitation of **luciferase** assays to fold induction seen in Northern blot analyses of RNA from treated cells, closely mimicked the induction behavior of the endogenous gene. Activation of gene expression occurred primarily with IFN-.beta. and double stranded RNA and to a lesser extent with other IFNs. This DNA sequence is therefore of considerable utility in understanding the **regulation** of mda-5 in particular and IFN-.beta.

inducible genes in general, also encompassing but not restricted to the analysis of compounds including synthetic small molecules that affect this pathway.

Detail Description Paragraph - DETX (204):

[0221] The complete intron/exon structure of the coding sequences has been determined. An approximately 6 kb fragment upstream of the transcription start site was also isolated. This fragment was cloned into a promoterless luciferase vector (pGL3 Basic, Promega) and assayed by transient transfection assays for transcriptional activity. The activity displayed by this promoter construct was identical to that of the endogenous gene in terms of responsiveness to inducers (recombinant human interferon or synthetic double stranded RNA, poly IC) and time kinetics of induction.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030092010 A1

TITLE: Molecular switches

PUBLICATION-DATE: May 15, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 996484

DATE FILED: November 28, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	PCT/GB00/02080	2000GB-PCT/GB00/02080	May 30, 2000
GB	9912635.1	1999GB-9912635.1	May 28, 1999
GB	0001582.6	2000GB-0001582.6	January 24, 2000

US-CL-CURRENT: 435/6, 435/7.1

ABSTRACT:

Provided herein are compositions comprising molecular switches and methods for identifying, selecting and using such molecular switches. Also provided are methods for the identification and use of ligand-dependent binding molecules and ligands.

----- KWIC -----

Summary of Invention Paragraph - BSTX (199):

[0195] Any changes that are made to the coding sequence can be made using techniques that are well known in the art and include site directed mutagenesis PCR, and synthetic gene construction. Such methods are described in published patent applications EP 0 385 962 (to Monsanto). EP 0 359 472 (to Lubrizol) and WO 93/07278 (to Ciba-Geigy). Well known protocols for transient expression in plants can be used to check the expression of modified genes before their transfer to plants by transformation.

Detail Description Paragraph - DETX (10):

[0352] In general, procedures and materials are in accordance with guidance given in Sambrook et al., Molecular Cloning. A Laboratory Manual, Cold Spring

Harbor, 1989. The **gene for the Zif268 fingers (residues 333420) is assembled from 8 overlapping synthetic** oligonucleotides (see Choo and Klug, (1994) PNAS (USA) 91:11163-67), giving SfiI and NotI overhangs. The genes for fingers of the phage library are synthesised from 4 oligonucleotides by directional end to end ligation using 3 short complementary linkers, and amplified by PCR from the single strand using forward and backward primers which contain sites for NotI and SfiI respectively. Backward PCR primers in addition introduce Met-Ala-Glu as the first three amino acids of the zinc finger peptides, and these are followed by the residues of the wild type or library fingers as required. Cloning overhangs are produced by digestion with SfiI and NotI where necessary. Fragments are ligated to 1 .mu.g similarly prepared Fd-Tet-SN vector. This is a derivative of fd-tet-DOG 1 (Hoogenboom et al., (1991) Nucleic Acids Res. 19,41334137) in which a section of the pelB leader and a restriction site for the enzyme SfiI (underlined) have been added by site-directed **mutagenesis** using the oligonucleotide:

Detail Description Paragraph - DETX (106):

[0430] To investigate the utility of heterologous zinc finger proteins for the **regulation** of plant **genes**, a **synthetic** zinc finger protein was designed and introduced into transgenic Arabidopsis thaliana under the control of a promoter capable of expression in a plant as described below. A second construct comprising the zinc finger protein binding sequence fused upstream of the Green Fluorescent Protein (**GFP**) reporter gene was also introduced into transgenic Arabidopsis thaliana as described in Example 8. Crossing the two transgenic lines produced progeny plants carrying both constructs in which the **GFP** reporter gene was expressed demonstrating transactivation of the gene by the zinc finger protein.



US-PAT-NO: 6645761

DOCUMENT-IDENTIFIER: US 6645761 B1

TITLE: Humanized polynucleotide sequence encoding Renilla  
mulleri green fluorescent protein

DATE-ISSUED: November 11, 2003

INVENTOR-INFORMATION:

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Vaillancourt; Peter Edward	Del Mar	CA	N/A	N/A

APPL-NO: 09/ 839650

DATE FILED: April 19, 2001

PARENT-CASE:

This application is a continuation-in-part of U.S. patent application Ser.  
No. 09/748,786, filed Dec. 22, 2000, now abandoned, incorporated herein by  
reference in its entirety.

US-CL-CURRENT: 435/325, 435/243 , 435/320.1 , 435/410 , 536/23.5

ABSTRACT:

The present invention provides a polynucleotide encoding a green fluorescent  
protein from Renilla mulleri comprising a humanized sequence which permits  
enhanced expression of the encoded polypeptide in mammalian cells.

3 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

----- KWIC -----

Brief Summary Text - BSTX (18):

The present invention further provides a method of producing R. mulleri GFP  
comprising the steps of: introducing a recombinant vector comprising a  
humanized polynucleotide sequence encoding R. mulleri GFP to a cell; culturing  
the cell; and isolating R. mulleri GFP from the cell.

Brief Summary Text - BSTX (21):

The present invention further provides a method of determining the location of a polypeptide of interest in a cell, the method comprising the steps of: linking said polynucleotide **sequence encoding a polypeptide of interest with a humanized polynucleotide encoding R. mulleri GFP, such that the linked polynucleotide sequences** are fused in frame; introducing the linked polynucleotide sequences to a cell; and determining the location of the polypeptide encoded by the linked polynucleotide sequences.

**Brief Summary Text - BSTX (26):**

The invention further provides a method of monitoring the activity of a transcriptional **regulatory** sequence, the method comprising the steps of: operably linking a nucleic acid **sequence comprising the transcriptional regulatory sequence to a humanized nucleic acid sequence** encoding R. mulleri **GFP** to form a reporter construct; introducing the reporter construct to a cell; and detecting R. mulleri **GFP** fluorescence in the cell, wherein the fluorescence reflects the activity of the transcriptional **regulatory** sequence.

**Brief Summary Text - BSTX (27):**

The invention still further provides a method of detecting a modulator of a transcriptional **regulatory** sequence, the method comprising the steps of: operably linking a nucleic acid **sequence comprising the transcriptional regulatory sequence to a humanized nucleic acid sequence** encoding R. mulleri **GFP** to form a reporter construct, wherein the transcriptional **regulatory** sequence is responsive to the presence of the modulator; introducing the reporter construct to a cell; and detecting R. mulleri **GFP** fluorescence in the cell, wherein the fluorescence indicates the presence of the modulator.

**Brief Summary Text - BSTX (28):**

The invention still further provides a method of screening for an inhibitor of a transcriptional **regulatory** sequence, the method comprising the steps of: operably linking a nucleic acid **sequence comprising the transcriptional regulatory sequence to a humanized nucleic acid sequence** encoding R. mulleri **GFP** to form a reporter construct; introducing the reporter construct to a cell; contacting the cell with a candidate inhibitor of the transcriptional **regulatory** sequence; and detecting R. mulleri **GFP** fluorescence in the cell, wherein a decrease in the fluorescence relative to that detected in the absence of the candidate inhibitor indicates that the candidate inhibitor inhibits the activity of the transcriptional **regulatory** sequence.

**Brief Summary Text - BSTX (29):**

The invention still further provides a method of producing a fluorescent molecular weight marker, the method comprising the steps of: linking a **humanized nucleic acid sequence** encoding R. mulleri **GFP** in frame to a nucleic acid sequence encoding a polypeptide of known relative molecular weight such that the linked molecules encode a fusion polypeptide; introducing the linked nucleic acid sequences to a cell; isolating said fusion polypeptide from the cell, wherein the fusion polypeptide is a relative molecular weight marker.

Brief Summary Text - BSTX (32):

In a still further embodiment, the humanized nucleic acid sequence encoding R. mulleri GFP is the sequence of SEQ ID NO: 1.

Brief Summary Text - BSTX (33):

The term "humanized R. mulleri polynucleotide" or "humanized R. mulleri GFP sequence" refers to a polynucleotide coding sequence in which at least 179 codons of the polynucleotide coding sequence for a non-human polypeptide (i.e., a polypeptide not naturally expressed in humans) have been altered to a codon sequence more preferred for expression in mammalian cells (i.e., SEQ ID NO: 1). In the "humanized R. mulleri GFP nucleotide sequence" of SEQ ID NO: 1, residue number 93 may be either a T or a C. In addition, an equivalent of a humanized sequence according to the invention is contemplated which is a polynucleotide according to SEQ ID NO: 1 in which one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty of those 179 codons that are altered to be humanized codons in SEQ ID NO: 1 are not altered such that they are humanized codons (that is, are not preferred in mammalian, particularly human, cells), provided expression in mammalian cells of the equivalent "humanized R. mulleri polynucleotide" described in SEQ ID NO: 1 is not reduced (relative to expression of the humanized sequence of SEQ ID NO: 1 in the same type of cells) by more than 5% or at most 10%.

Brief Summary Text - BSTX (34):

The amount of fluorescent polypeptide expressed in a human cell from a humanized GFP polynucleotide sequence is at least two-fold greater, on either a mass or a fluorescence intensity scale per cell, than the amount expressed from an equal amount or number of copies of a wild type R. mulleri GFP polynucleotide.

Brief Summary Text - BSTX (35):

As used herein, the term "humanized codon" means a codon, within a polynucleotide sequence encoding a non-human polypeptide, that has been changed to a codon that is more preferred for expression in human cells relative to that codon encoded by the non-human organism from which the non-human polypeptide is derived. Species-specific codon preferences stem in part from differences in the expression of tRNA molecules with the appropriate anticodon sequence. That is, one factor in the species-specific codon preference is the relationship between a codon and the amount of corresponding anticodon tRNA expressed.

Drawing Description Text - DRTX (2):

FIG. 1 shows the coding sequence of humanized R. mulleri GFP, SEQ ID NO: 1. Residue number 93 can be T or C.

Drawing Description Text - DRTX (5):

FIG. 4 shows a sequence alignment between non-humanized and humanized polynucleotide sequences encoding R. mulleri GFP. Vertical lines represent homology between the humanized and non-humanized genes. Gaps represent nucleotides that were altered to produce the hmGFP gene (i.e., the difference between SEQ ID NO: 1 and SEQ ID NO: 2). The valine at position 2 in the hmGFP sequence was inserted to accommodate an optimal Kozak translation initiation sequence.

Detailed Description Text - DETX (2):

The invention is based upon the discovery of a humanized polynucleotide sequence encoding R. mulleri GFP.

Detailed Description Text - DETX (3):

Also disclosed herein are methods of using a humanized R. mulleri GFP gene to produce an R. mulleri GFP polypeptide, the methods comprising introducing an expression vector containing a humanized coding sequence for R. mulleri GFP into a cell, culturing the cell, and isolating the GFP polypeptide.

Detailed Description Text - DETX (5):

A number of methodologies were combined to provide the invention disclosed herein, including molecular, cellular and biochemical approaches. Polynucleotides encoding R. mulleri GFP or a variant GFP sequence to which a humanized sequence is desired are obtained in any of several different ways known to those of skill in the art, including direct chemical synthesis, library screening and PCR amplification. A. Polynucleotide sequence encoding wild type R. mulleri GFP.

Detailed Description Text - DETX (18):

The present invention provides a modified nucleic acid sequence which represents a humanized form of R. mulleri which provides of enhanced expression of the encoded GFP polypeptide in human cells. To generate a humanized polynucleotide encoding R. mulleri GFP, useful in the present invention, the nucleic acid sequence encoding the polypeptide may be modified to enhance its expression in mammalian or human cells. The codon usage of R. mulleri is optimal for expression in R. mulleri, but not for expression in mammalian or human systems. Therefore, the adaptation of the sequence isolated from the sea pansy for expression in higher eukaryotes involves the modification of specific codons to change those less favored in mammalian or human systems to those more commonly used in these systems. This so-called "humanization" is accomplished by site-directed mutagenesis of the less favored codons as described herein below or as known in the art. The preferred codons for human gene expression are listed in Table 1. The codons in the table are arranged from left to right in descending order of relative use in human genes.

Detailed Description Text - DETX (19):

Humanized nucleotide sequences encoding R. mulleri may be generated by site directed mutagenesis. The humanized nucleotide sequences of SEQ ID NO: 1 may,

of course, be varied slightly by altering several humanized codons to be non-preferential codons in a mammalian or human cell and such slight alterations are considered to be equivalent as long as they do not reduce the level of expression of the humanized gene in mammalian cells by more than 5 or 10% relative to the expression of the sequence of SEQ-ID NO: 1.

Detailed Description Text - DETX (20):

There are 64 possible combinations of the 4-DNA nucleotides in codon groups of 3, and the genetic code is redundant for many of the 20 amino acids. Each of the different codons for a given amino acid encodes the incorporation of that amino acid into a polypeptide. However, within a given species there tends to be a preference for certain of the redundant codons to encode a given amino acid. The "codon preference" of R. mulleri is different from that of humans (this codon preference is usually based upon differences in the level of expression of the tRNAs containing the corresponding anticodon sequences). Table 1 shows the preferred codons for human gene expression. A codon sequence is preferred for human expression if it occurs to the left of a given codon sequence in the table. Optimally, but not necessarily, less preferred codons in a non-human polynucleotide coding sequence are humanized by altering them to the codon most preferred for that amino acid in human gene expression.

Detailed Description Text - DETX (22):

The production of R. mulleri GFP polypeptides (e.g., the polypeptide with the amino acid sequence of SEQ ID NO: 2) from recombinant vectors comprising humanized GFP-encoding polynucleotides of the invention may be effected in a number of ways known to those skilled in the art. For example, plasmids, bacteriophage or viruses may be introduced to prokaryotic or eukaryotic cells by any of a number of ways known to those skilled in the art. Following introduction of R. mulleri GFP-encoding polynucleotides to a prokaryotic or eukaryotic cell, expressed GFP polypeptides may be isolated using methods known in the art or described herein below. Useful vectors, cells, methods of introducing vectors to cells and methods of detecting and isolating GFP polypeptides are also described herein below.

Detailed Description Text - DETX (44):

Any plasmid vector that allows expression of a humanized GFP coding sequence of the invention in a selected host cell type is acceptable for use according to the invention. A plasmid vector useful in the invention may have any or all of the above-noted characteristics of vectors useful according to the invention. Plasmid vectors useful according to the invention include, but are not limited to the following examples: Bacterial--pQE70, pQE60, pQE-9 (Qiagen) pBs, phagescript, psiX174, pBluescript SK, pBskS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia); Eukaryotic--pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia). However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

Detailed Description Text - DETX (48):

A number of different viral vectors are useful according to the invention, and any viral vector that permits the introduction and expression of humanized sequences encoding R. mulleri GFP thereof in cells is acceptable for use in the methods of the invention. Viral vectors that can be used to deliver foreign nucleic acid into cells include but are not limited to retroviral vectors, adenoviral vectors, adeno-associated viral vectors, herpesviral vectors, and Semliki forest viral (alphaviral) vectors. Defective retroviruses are well characterized for use in gene transfer (for a review see Miller, A. D. (1990) Blood 76:271). Protocols for producing recombinant retroviruses and for infecting cells in vitro or in vivo with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F. M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14, and other standard laboratory manuals.

Detailed Description Text - DETX (51):

2. Host Cells Useful According to the Invention. Any cell into which a recombinant vector carrying a gene encoding R. mulleri GFP or humanized version may be introduced and wherein the vector is permitted to drive the expression of the GFP is useful according to the invention. That is, because of the wide variety of uses for the GFP molecules of the invention, any cell in which a GFP molecule of the invention may be expressed and preferably detected is a suitable host, wherein the host cell is preferably a mammalian cell and more preferably a human cell. Vectors suitable for the introduction of GFP-encoding sequences to host cells from a variety of different organisms, both prokaryotic and eukaryotic, are described herein above or known to those skilled in the art.

Detailed Description Text - DETX (71):

Humanized polynucleotide sequences encoding R. mulleri GFP are useful in a number of different ways. Generally, a polynucleotide sequence encoding R. mulleri GFP is useful in any process or assay that can be performed with A. victoria GFP. Further, because of its enhanced expression in mammalian cells and fluorescent intensity, a humanized polynucleotide sequence encoding R. mulleri GFP is useful in processes and assays beyond those that can be performed with A. victoria GFP.

Detailed Description Text - DETX (72):

Humanized polynucleotide sequences encoding R. mulleri GFP may be used as selectable markers for the identification of cells transfected or infected with a gene transfer vector. In this aspect, cells transfected with a humanized construct encoding GFP may be identified over a background of non-transfected or infected cells by illumination of the cells with light within the excitation spectrum and detection of fluorescent emission in the emission spectrum of the GFP.

Detailed Description Text - DETX (73):

Humanized R. mulleri GFP genes can be used to identify transformed mammalian cells (e.g., by fluorescence-activated cell sorting (FACS) or fluorescence microscopy), particularly human cells, to measure gene expression in vitro and

in vivo, to label specific cells in multicellular organisms (e.g., to study cell lineages), to label and locate fusion proteins, and to study intracellular protein trafficking.

Detailed Description Text - DETX (77):

A humanized polynucleotide sequence encoding R. mulleri GFP may be introduced as a selectable marker to identify transfected mammalian cells from a background of non-transfected cells. Alternatively, humanized R. mulleri GFP transfection may be used to pre-label isolated cells or a population of similar cells prior to exposing the cells to an environment in which different cell types are present. Detection of GFP in only the original cells allows the location of such cells to be determined and compared with the total population.

Detailed Description Text - DETX (78):

Mammalian cells that have been transfected with exogenous DNA can be identified with polynucleotide sequence encoding R. mulleri GFPs of the invention without creating a fusion protein. The method relies on the identification of cells that have received a plasmid or vector that comprises at least two transcriptional or translational units. A first unit will encode and direct expression of the desired protein, while the second unit will direct expression of humanized polynucleotide sequences encoding R. mulleri GFP. Co-expression of GFP from the second transcriptional or translational unit ensures that cells containing the vector are detected and differentiated from cells that do not contain the vector.

Detailed Description Text - DETX (79):

The humanized R. mulleri GFP sequences of the invention may also be fused to a DNA sequence encoding a selected protein in order to directly label the encoded protein with GFP. Expressing such an R. mulleri GFP fusion protein in a human cell results in the production of fluorescently-tagged proteins that can be readily detected. This is useful in confirming that a protein is being produced by a chosen host cell. It also allows the location of the selected protein to be determined, whether this represents a natural location or whether the protein has been artificially targeted to another location.

Detailed Description Text - DETX (80):

B. Use of humanized polynucleotides encoding R. mulleri for analysis of transcriptional regulatory sequences.

Detailed Description Text - DETX (81):

The humanized R. mulleri GFP genes of the invention allow a range of transcriptional regulatory sequences to be tested for their suitability for use with a given gene, cell, or system, but preferably for use with mammalian cells, preferably human cells. This applies to in vitro uses, such as in identifying a suitable transcriptional regulatory sequence for use in recombinant expression and high level protein production, as well as in vivo uses, such as in pre-clinical testing or in gene therapy in human subjects.

Detailed Description Text - DETX (84):

The humanized GFP encoded by a regulatory sequence testing construct may optionally have a secretion signal fused to it, such that GFP secreted to the medium is detected.

Detailed Description Text - DETX (86):

C. Use of humanized polynucleotide sequences encoding R. mulleri GFP in assays for compounds that modulate transcription.

Detailed Description Text - DETX (87):

Humanized polynucleotide sequences encoding R. mulleri GFP are useful in screening assays to detect compounds that modulate transcription. In this aspect of the invention, humanized R. mulleri GFP coding sequences are positioned downstream of a promoter that is known to be inducible by the agent that one wishes to detect. Expression of GFP in the cells will normally be silent, and is activated by exposing the cell to a composition that contains the selected agent. In using a promoter that is responsive to, for example, a lipid soluble transcriptional modulator, a toxin, a hormone, a cytokine, a growth factor or other defined molecule, the presence the particular defined molecule can be determined. For example, an estrogen-responsive regulatory sequence may be linked to GFP in order to test for the presence of estrogen in a sample.

Detailed Description Text - DETX (89):

D. Use of humanized polynucleotide sequences encoding R. mulleri GFP in FACS analyses.

Detailed Description Text - DETX (91):

As with other expression embodiments, a desired protein may be directly labeled with GFP by preparing a fusion protein comprising a humanized polynucleotide sequence encoding GFP for expression in a cell; preferably a humanized GFP fusion protein in a human cell. A humanized polynucleotide sequence encoding GFP can also be co-expressed from a second transcriptional or translational unit within the expression vector that expresses desired protein, as described above. Cells expressing the GFP-tagged protein or cells co-expressing GFP are then detected and sorted by FACS analysis.

Detailed Description Text - DETX (92):

F. Other uses of humanized polynucleotide sequences encoding R. mulleri GFP fusion proteins.

Detailed Description Text - DETX (93):

Humanized R. mulleri GFP genes can be used as one portion of a fusion protein, allowing the location of the tagged protein to be identified. Fusions



of GFP with an exogenous protein should preserve both the fluorescence of GFP and functions of the host protein, such as physiological functions and/or targeting functions.

Detailed Description Text - DETX (98):

Comparison of Expression of Humanized Versus Wild Type Genes Encoding R. mulleri GFP

Detailed Description Text - DETX (99):

The humanized R. mulleri GFP coding sequence can be tested for expression in several human, rodent and monkey cell lines. Fluorescence levels are expected to be substantially higher for the humanized rGFP (hrGFP) gene compared with that for rGFP. In a direct comparison between cell populations harboring single copy proviral expression cassettes encoding either hrGFP or the humanized, red-shifted Aequorea GFP (EGFP), relative fluorescence intensity is expected to be comparable between the two genes.

Detailed Description Text - DETX (108):

To confirm enhanced expression of a humanized R. mulleri GFP nucleic acid sequence in human cells, nucleic acid encoding the humanized sequence was expressed in human HeLa cells. Production of viral particles encoding the humanized GFP for transduction of human cells was carried out by co-transfecting 293 cells with 3 .mu.g each of the retroviral packaging vectors pVPack-GP, pVPack-VSV-G (Stratagene) and pCFB-hmGFP (humanized R. mulleri GFP; FIG. 6). The transfections were carried out according to Pear et al. (1997, Methods in Molecular Medicine: Gene Therapy Protocols, Robbind (Ed.) Humana Press, Totawa, N.J.), but modified by using the MBS Transfection Kit (Stratagene). Subsequently, 2.times.10.sup.5 HeLa cells were infected with tissue culture supernatant containing no virus (FIG. 7 gray curve) or containing virus prepared using pCFB-hmGFP (FIG. 7, black curve). After 72 hours, cells were trypsinized and analyzed by FACS (Cytometry Research Services, Sorrento Valley, Calif.) using standard FITC filters (FIG. 7).

Detailed Description Text - DETX (110):

To confirm that the fluorescence spectra for the cloned, humanized gene encoding R. mulleri GFP is identical to that previously reported for the native protein, the fluorescence spectra of human cells expressing the humanized GFP was examined. HeLa cells transduced with the hmGFP-expressing retrovirus, described above, were lysed in PBS by three cycles of freeze-thawing using liquid nitrogen and a 37.degree. C. water bath. The lysates were cleared by high-speed centrifugation, and the supernatants were then used for spectral analysis. Excitation and emission spectral analysis was determined using a Shimadzu RF-1501 Spectrofluorophotometer. Excitation and emission scans were performed on equal amounts of total protein prepared from transfected or untransfected HeLa cells. Background fluorescence was subtracted from the scans of the GFP-containing (transfected) extract by normalization to the scans of the untransfected extracts. FIG. 8 shows that the fluorescence spectra of cell extracts containing hmGFP is the same as that for native R. mulleri GFP.

with the major excitation peak at 500 nm and the major emission peak at 506 nm.

Claims Text - CLTX (1):

1. A humanized polynucleotide encoding R. mulleri GFP comprising the sequence of SEQ ID NO: 1, wherein the nucleotide at position 93 is either a T or C.

US-PAT-NO: 6639050

DOCUMENT-IDENTIFIER: US 6639050 B1

TITLE: Synthetic genes for plant gums and other  
hydroxyproline-rich glycoproteins

DATE-ISSUED: October 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kieliszewski; Marcia J.	Albany	OH	N/A	N/A

APPL-NO: 09/ 547693

DATE FILED: April 12, 2000

PARENT-CASE:

This is a continuation-in-part of co-pending application Ser. No. 09/119,507 filed on Jul. 20, 1998 which is a continuation-in-part of co-pending application Ser. No. 08/897,556 filed on Jul. 21, 1997.

US-CL-CURRENT: 530/322, 435/252.3, 435/252.33, 435/320.1, 435/410  
, 435/419, 530/326, 530/327, 530/344, 530/370, 536/23.6

ABSTRACT:

A new approach in the field of plant gums is described which presents a new solution to the production of hydroxyproline(Hyp)-rich glycoproteins (HRGPs), repetitive proline-rich proteins (RPRPs) and arabinogalactan-proteins (AGPs). The expression of synthetic genes designed from repetitive peptide sequences of such glycoproteins, including the peptide sequences of gum arabic glycoprotein (GAGP), is taught in host cells, including plant host cells.

7 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

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Detailed Description Text - DETX (157):

Plasmids pBI121-Sig and pBI121-Sig-EGFP are constructed as follows. For both plasmids, the GUS gene present in pBI121 (Clontech) is deleted by digestion with BamHI and SstI and a pair of partially complementary oligonucleotides encoding the tobacco extensin signal sequence is annealed to

the BamHI and SstI ends. The oligonucleotides encoding the 21 amino acid extensin signal sequence have the following sequence: 5'-GA TCC GCA ATG GGA AAA ATG GCT TCT CTA TTT GCC ACA TTT TTA GTG GTT TTA GTG TCA CTT AGC TTA GCA CAA ACA ACC CGG GTA CCG GTC GCC ACC ATG GTG TAA AGC GGC CGC GAG CT-3' (SEQ ID NO:98) and 5'-C GCG GCC GCT TTA CAC CAT GGT GGC GAC CGG TAC CCG GGT TGT TTG TGC TAA GCT AAG TGA CAC TAA AAC CAC TAA AAA TGT GGC AAA TAG AGA AGC CAT TTT TCC CAT TGC G-3' (SEQ ID NO:99). In addition to encoding the extensin signal sequence, this pair of oligonucleotides, when inserted into the digested pBI121 vector, provides a BamHI site (5' end) and XmaI and SstI sites (3' end). The XmaI and SstI sites allow the insertion of the **GFP** gene. The modified pBI121 vector lacking the GUS gene and containing the synthetic extensin signal sequence is termed pBI121-Sig. Proper construction of pBI121 is confirmed by DNA sequencing.

#### Detailed Description Text - DETX (160):

Expression of the synthetic HRGP gene cassettes is not dependent upon the use of the pBI121-Sig and pBI121-Sig-EGFP gene cassette. Analogous expression vectors containing other promoter elements functional in plant cells may be employed (e.g., the CaMV region IV promoter, ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu) promoter, the nopaline promoter, octopine promoter, mannopine promoter, the .beta.-conglycinin promoter, the ADH promoter, heat shock promoters, tissue-specific promoters, e.g., promoters associated with fruit ripening, promoters regulated during seed ripening (e.g., promoters from the napin, phaseolin and glycinin genes). For example, expression vectors containing a promoter that directs high level expression of inserted gene sequences in the seeds of plants (e.g., fruits, legumes and cereals, including but not limited to corn, wheat, rice, tomato, potato, yam, pepper, squash cucumbers, beans, peas, apple, cherry, peach, black locust, pine and maple trees) may be employed. Expression may also be carried out in green algae.

#### Detailed Description Text - DETX (161):

In addition, alternative reporter genes may be employed in place of the **GFP** gene. Suitable reporter genes include .beta.-glucuronidase (GUS), neomycin phosphotransferase II gene (nptII), alkaline phosphatase, luciferase, CAT (Chloramphenicol AcetylTransferase). Preferred reporter genes lack Hyp residues. Further, the proteins encoded by the synthetic HRGP genes need not be expressed as fusion proteins. This is readily accomplished using the pBI121-Sig vector.

#### Detailed Description Text - DETX (166):

An expression vector containing the synthetic GAGP gene cassette (capable of being expressed as a fusion with **GFP** or without **GFP** sequences) is introduced into tomato cell suspension cultures. A variety of means are known to the art for the transfer of DNA into tomato cell suspension cultures, including

Agrobacterium-mediated transfer and biolistic transformation.

Detailed Description Text - DETX (168):

Transgenic cells/plants can be examined for transgene copy number and construct fidelity genomic Southern blotting and for the HRGP construct mRNA by northern blotting, using the internal repeat oligonucleotides as probes. Controls include tissue/plants which are untransformed, transformed with the pBI121 alone, pBI121 containing only GFP, and pBI121 having the signal sequence and GFP but no HRGP synthetic gene.

Detailed Description Text - DETX (306):

Plasmid pUC18 has an endogenous BsrFI site in the Amp.sup.r gene. This site was eliminated by mutation to make the plasmid amenable to subcloning of the XmaI-BsrFI synthetic gene fragments, using the PCR core system I kit (Promega). The PCR Primer 1: (upstream primer) had the sequence (SEQ ID NO:204) GATACGCGAGACCCACGCTC ACCAGTCC; this primer was designed from nucleotides 1756

to 1785 of pUC18 except for 1 substitution (A for G) at position 1780 (bolded and underlined). This changes one Ala codon (GCC) for another (GCT), retaining the Amp.sup.r amino acid sequence while mutating the BsrFI site. PCR Primer 2: (downstream primer) had the sequence (SEQ ID NO:205) CTCGGTCGCCGCATACACTAT and was designed from nt 2220 to 2198 of pUC18. The PCR reaction conditions were 2 min @ 95.degree. C., 30 sec @ 95.degree. C., 1 min @ 48.degree. C., 1 min @ 72.degree. C. (30 cycles), 5 min @ 72.degree. C. PCR products were separated on a 1.5% agarose gel. The 464 bp PCR fragment was extracted from the gel using the QIAEX II gel extraction kit. The isolated fragment was restricted and subcloned into pUC18 as a ScaI-BpmI fragment. The new plasmid was designated MpUC18 and has an active Amp.sup.r gene and no BsrFI site.

Detailed Description Text - DETX (313):

The next subcloning was done to put the EGFP gene in frame behind the gum sequences. pUC ss-EGFP plasmid was cut with XmaI and treated with Mung Bean endonuclease (New England Biolabs). The enzymes were inactivated by phenol/chloroform extraction followed by ethanol precipitation. Then plasmid was cut with SacI. The EGFP fragment isolated after restriction was subcloned into pUC ss-gum plasmids which was cut with SmaI/SacI restriction enzymes. The signal sequence-synthetic gene-EGFP fragments were removed from MpUC18 plasmid as BamHI/SacI fragments and subcloned into pBI121, replacing the .beta.-glucuronidase reporter gene. The MpUC ss-gum.sub.20 -EGFP and MpUC ss-gum.sub.32 -EGFP plasmids were sequenced with pUC/M13 forward (17 mer) primer and with GFP primer GAAGATGGTGCGCTCCTGGACGT (SEQ ID NO:226) from nucleotide 566 to nucleotide 588 of pEGFP.

US-PAT-NO: 6596539

DOCUMENT-IDENTIFIER: US 6596539 B1

TITLE: Modification of virus tropism and host range by viral genome shuffling

DATE-ISSUED: July 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer; Willem P. C.	Los Altos	CA	N/A	N/A
Patten; Phillip	Menlo Park	CA	N/A	N/A
Soong; Nay Wei	Sunnyvale	CA	N/A	N/A

APPL-NO: 09/ 183037

DATE FILED: October 30, 1998

PARENT-CASE:

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation-in-part of 08/962,236 filed Oct. 31, 1997 now abandoned. The present application claims benefit of the 08/962,236 application, which is incorporated herein by reference in its entirety for all purposes.

US-CL-CURRENT: 435/440, 435/455 , 435/456 , 435/457 , 435/5 , 435/6

ABSTRACT:

The invention relates to a method and compositions for modifying a phenotype of a virus, such as viral tropism and host range, by iterative sequence recombination of variant viruses and selection of improved variants.

16 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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Detailed Description Text - DETX (92):

Transgenes and expression vectors can be constructed by any suitable method known in the art. It is often desirable to generate coding sequences for CD4, CCR5, CXCR4, and other human accessory proteins that aid viral infectivity by

either PCR or RT-PCR amplification from a suitable human cell type (e.g., a T lymphocyte population) or by ligating or amplifying a set of overlapping synthetic oligonucleotides: publicly available sequence databases and the literature can be used to select the polynucleotide sequence(s) to encode the specific protein desired, including any mutations, consensus sequence, or mutation kernel desired by the practitioner. The coding sequence(s) are operably linked to a transcriptional regulatory sequence (e.g., T cell lineage-specific promoter/enhancer) and, if desired, an origin of replication (e.g., EBV ori) for episomal replication, or one or more flanking sequences having substantial sequence identity to a host chromosomal sequence to provide for homologous recombination and targeted integration of the transgene. In an embodiment, a transgene comprises a human CD4 minigene or a substantially complete human CD4 gene. Similar transgenes comprise a CCR5 and/or CXCR4 minigene or substantially complete gene. The transgenes can use the native gene transcriptional regulatory sequences, or can employ an operably linked heterologous transcriptional regulatory sequence (e.g., a mouse CD4 promoter/enhancer, a CMV promoter/enhancer, a human T cell receptor gene promoter/enhancer, and the like). Often the transgene(s) and expression vector(s) will further comprise a reporter gene or a selectable marker gene (e.g., tk, neo) in a selection cassette to facilitate identification and enrichment of cells having the construct in functional form.

#### Detailed Description Text - DETX (315):

In one embodiment, the following steps are used to evolve HIV for replication in non-human cells. First, cloning vectors and protocols for shuffling infectious molecular clones in two non-infectious pieces are established. Second, methods for efficiently making large (>10<sup>6</sup> complexity) libraries of infectious molecules from shuffled fragments of HIV-1 are established. Third, libraries of HIV-1 recombinants are produced using in vivo recombination pathways. Fourth, synthetic HIV-1 tat genes representing all clades are bred into infectious virus and make large libraries of infectious molecular clones, based on the techniques established in the first and second step. Fifth, CD4<sup>+</sup> CCR5<sup>+</sup> mouse cells are infected with these libraries and selected for mutants which can replicate and be passaged in tissue culture. Eight consensus tat genes representing all eight HIV-1 clades were synthesized. Sixth, mouse and human reporter cells containing constructs with GFP under the control of an HIV-1 LTR were constructed. Seventh, the transcriptional activation activities of the synthetic tat genes in the reporter cells are validated. Ninth, libraries of shuffled tat genes are constructed in an MLV retroviral vector and introduce them into the reporter cells. Tenth, methods to FACS select libraries for cells with tat induced GFP are established, and the tat genes recovered by PCR, shuffled, and reconstruct new libraries of shuffled genes. Eleventh, evolved tat genes are bred into the context of infectious molecular clones and select for growth on mouse cells. For Macaques, an immortalized macaque cell line is constructed expressing macaque CD4 and CCR5, which are known to support efficient entry of HIV into macaque cells. This cell line is used to select in tissue culture for replication of HIV in macaque cells.

US-PAT-NO: 6573099

DOCUMENT-IDENTIFIER: US 6573099 B2

TITLE: Genetic constructs for delaying or repressing the  
expression of a target gene

DATE-ISSUED: June 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Graham; Michael Wayne	St. Lucia	N/A	N/A	AU

APPL-NO: 09/ 100812

DATE FILED: June 19, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
AU	PP2492	March 20, 1998

US-CL-CURRENT: 435/455, 424/93.2 , 424/93.21 , 435/320.1 , 435/325 , 514/44  
, 536/24.5

ABSTRACT:

The present invention relates generally to synthetic genes for modifying endogenous gene expression in a cell, tissue or organ of a transgenic organism, in particular a transgenic animal or plant. More particularly, the present invention provides novel synthetic genes and genetic constructs which are capable of repressing delaying or otherwise reducing the expression of an endogenous gene or a target gene in an organism when introduced thereto.

22 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 28

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Brief Summary Text - BSTX (7):

In work leading up to the present invention, the inventors sought to elucidate the mechanisms involved in down-regulating gene expression in an attempt to provide improved methods therefor. In so doing the inventors have developed a wide range of synthetic genes capable of modulating gene expression in both prokaryotic and eukaryotic cells and genetic constructs comprising same.



Detailed Description Text - DETX (5):

The term "**synthetic gene**" refers to a non-naturally occurring gene as hereinbefore defined which preferably comprises at least one or more transcriptional and/or translational **regulatory** sequences operably linked to a structural gene sequence.

Detailed Description Text - DETX (13):

Generally, a gene of the invention may be subjected to **mutagenesis** to produce single or multiple nucleotide substitutions, deletions and/or additions without affecting its ability to modify target gene expression. Nucleotide insertional derivatives of the **synthetic gene** of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product.

Detailed Description Text - DETX (23):

Alternatively, the structural gene may comprise a nucleotide sequence which does not encode an amino acid sequence or more commonly, comprises one or more open reading frames which encode one or more peptides, oligopeptides or polypeptides which are unrelated at the amino acid sequence level to the amino acid sequence encoded by the target gene. For example, the mRNA product of the structural **gene may be inserted into the synthetic gene** of the invention so as to alter or disrupt the reading frame of the structural gene and produce one or more frame shift **mutations** in the translation product thereof relative to the translation product encoded by the target gene, notwithstanding a substantial identity between the structural gene and the target gene on the one hand and the corresponding mRNA products of the structural gene and the target gene on the other hand. Such effects may be produced by introducing one or two nucleotide substitutions or deletions into the structural gene, relative to the target gene sequence or alternatively, by introducing a translation start codon 5'-ATG-3' upstream of any nucleotide in the structural gene which occurs at a particular position in a codon of the corresponding target gene such that the position of said nucleotide in the codon of the structural gene is altered.

Detailed Description Text - DETX (27):

A promoter is usually, but not necessarily, positioned upstream or 5', of the structural **gene component of the synthetic gene** of the invention, the expression of which it **regulates**. Furthermore, the **regulatory** elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the structural gene.

Detailed Description Text - DETX (30):

Examples of promoters suitable for use in the **synthetic genes** of the present invention include viral, fungal, bacterial, animal and plant derived promoters

capable of functioning in plant, animal, insect, fungal, yeast or bacterial cells. The promoter may **regulate** the expression of the structural gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or pathogens, or metal ions, amongst others.

Detailed Description Text - DETX (167):

**Synthetic Genes** and Genetic Constructs Comprising the lacI and Green Fluorescent Protein (**GFP**) Open Reading Frames

Detailed Description Text - DETX (172):

**Synthetic Genes** and Genetic Constructs Comprising the lacI and Green Fluorescent Protein (**GFP**) and Tyrosinase Open Reading Frames

US-PAT-NO: 6541197

DOCUMENT-IDENTIFIER: US 6541197 B2

TITLE: Vehicles for stable transfer of green fluorescent protein gene and methods of use for same

DATE-ISSUED: April 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Link, Jr.; Charles J.	Des Moines	IA	N/A	N/A
Levy; John P.	West Des Moines	IA	N/A	N/A
Wang; Suming	Des Moines	IA	N/A	N/A
Seregina; Tatiana	West Des Moines	IA	N/A	N/A

APPL-NO: 08/ 786531

DATE FILED: January 21, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of provisional application Serial. No. 60/010,371 filed Jan. 22, 1996.

US-CL-CURRENT: 435/5, 435/320.1, 435/325, 435/350, 435/351, 435/352, 435/366, 435/4, 435/6

ABSTRACT:

The present invention describes an efficient retroviral or viral based method that allows easy and quick identification of gene transfer in living, transduced mammalian cells. Retroviral and viral vector producer cells were generated containing a gene for an improved humanized red-shifted, Green Fluorescent Protein (hRGFP) which increases the resulting fluorescence yield after excitation. This humanized, red-shifted GFP (hRGFP) gene was cloned into several vectors and transfected into various packaging cell lines to produce vibrant green fluorescence after excitation with blue light at 450-490 nm. These vectors represent a substantial advance over currently available gene transfer marking systems or wild-type GFP marker systems none of which have been stably transfected into cells.

5 Claims, 66 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 55

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Abstract Text - ABTX (1):

The present invention describes an efficient retroviral or viral based method that allows easy and quick identification of gene transfer in living, transduced mammalian cells. Retroviral and viral vector producer cells were generated containing a **gene for an improved humanized** red-shifted, Green Fluorescent Protein (hRGFP) which increases the resulting fluorescence yield after excitation. This **humanized, red-shifted GFP (hRGFP) gene** was cloned into several vectors and transfected into various packaging cell lines to produce vibrant green fluorescence after excitation with blue light at 450-490 nm. These vectors represent a substantial advance over currently available gene transfer marking systems or wild-type **GFP** marker systems none of which have been stably transfected into cells.

Brief Summary Text - BSTX (9):

A novel marker gene is now available that will alleviate these cumbersome and time consuming steps for detecting gene transfer. The Green Fluorescent Protein (**GFP**) is a vibrant green bioluminescent marker which offers outstanding properties. The **gene has been sequenced, humanized** and is commercially available through several sources, however there has been much difficulty in finding a suitable transformation vehicle that will give stable expression in mammalian cells.

Brief Summary Text - BSTX (16):

The present invention describes the cloning and characterization of amphotropic retroviral vectors capable of demonstrating efficient, stable transfer of **humanized, red shifted GFP (hRGFP) gene** into mammalian cells. Living cells transfected and/or transduced with hRGFP have a stable, bright green fluorescence after excitation with blue light.

Drawing Description Text - DRTX (4):

FIG. 2 shows the retroviral constructs containing the red shift, humanized Green Fluorescent Protein. The wild-type **GFP and the humanized, red shifted GFP gene** were cloned into the pLNCX retroviral backbone. Plasmid pLNCG was constructed by PCR amplification of a wild-type **GFP** containing DNA fragment and subsequent subcloning into pLNCX. LTR, long terminal repeat; pA, polyadenylation signal; arrows indicate transcriptional start sites; .PSI..sup.+ indicates the presence of the viral packaging **sequence; GFP, wild-type green fluorescent protein; hRGFP, humanized, red shifted GFP.**

Detailed Description Text - DETX (4):

As used herein, "nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. Generally, DNA segments encoding the proteins provided by this invention are assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a **synthetic gene**

which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

Detailed Description Text - DETX (29):

The key to expression seems to lie in the 3 amino acid residues which form a cyclized chromophore. Specifically, the serine at position 65 is a site at which several amino acid replacements show increased intensity and quicken the rate of chromophore formation. The red shift may be responsible for advancing expression to this level. However, humanizing of the coding sequences seems to be providing the dominant contribution. Jellyfish are extremely divergent from mammals and consequently have different codon usage. This may present a translation challenge for mammalian cells of enough proportion to prevent a build up of detectable GFP. The HGS65T GFP gene contains 169 codon changes (Clontech) representing 71% of the gene. The above results show that this is a superior marker gene to follow retroviral and viral transduction.

Detailed Description Text - DETX (31):

In vivo retroviral transduction experiments may also take advantage of this marker system. PA317-HGS65T VPC cells injected into an established subcutaneous tumor cell will transduce the dividing tumor cells. VPC's used in black mice systems will be destroyed by the immune system within 2 weeks after injection. The F.A.C.S. sorting capabilities also opens up some doors of opportunity for leukemia trials. Overall, this evidence demonstrated that humanized red shift GFP has the potential of becoming a major player in gene therapy.

Detailed Description Text - DETX (32):

The instant invention demonstrates the effectiveness of a humanized, red shifted mutant GFP by retroviral and viral mediated gene transfer into human tumor cells and murine fibroblasts. A few molecular genetics groups have now reported mutations of the wild-type GFP gene which can generate GFP gene products with modified excitation and emission spectra. See for e.g. Heim, R., et al. (1994) Proc Natl Acad Sci. USA 91:12501-12504. The longer wavelength excitation peak (475 nm) of native A. Victoria GFP has lower amplitude than its shorter wavelength excitation peak (470-490 nm) with fluorescence amplitudes from 4-6 fold greater than from the wild-type gene product. Heim, R. (1995) Nature 373:663-664. Interestingly, this mutant also had more rapid formation of the fluorochrome. Id. Furthermore, the mutated, red shifted GFP had its codons modified to usage common in mammals (Dr. Sergei Zolotukhi and Dr. Nicholas Muzyczka, University of Florida, unpublished results). The inventors have evaluated this humanized version of a serine-65 to threonine codon mutant that demonstrates excitation at 490 nm and emissions at 510 nm in current gene transfer experiments. Comparisons between the wild-type GFP and the humanized, serine-65 red shifted mutant (hRGFP) demonstrated substantial improvement in fluorescence expression after either transfection and retroviral mediated GFP gene transfer (Table 1).

Detailed Description Text - DETX (46):

Plasmid preparation and digoxin probes. Plasmid pGFP-Cl containing wild-type **GFP** was obtained from Clontech (Palo Alto, Calif.). The plasmid pTR-Uf2 containing the **humanized red, shifted GFP (hRGFP) gene** was kindly provided by Dr. Sergei Zolotukhin and Dr. Nicholas Muzyczka (University of Florida). Construct plasmid DNA was transformed into DH5a competent cells and colonies grown on L-broth supplemented with ampicillin (50 .mu.g/ml) plates (LB/AMP) and transferred onto nylon membranes. The membrane was probed with a Dig-**GFP** probe using a digoxin probe kit (Boehringer-Mannheim). Primers for the Dig-**GFP** probe amplification of a **GFP** fragment were 5' primer 5' GGG AAG CTT TTA TTA TTT GTA TAG TTC ATC CAT GCC (SEQ ID NO:7) and 3' primer 5' GGG AAG CTT GCG CGT ATG GGT AAA GGA GAA GAA CTT (SEQ ID NO:8). Positive colonies were grown up in LB/AMP broth and plasmid DNA was isolated using the Qiagen plasmid prep kits (Qiagen Corp., Chatsworth, Calif.).

#### Detailed Description Text - DETX (56):

These results demonstrate the effectiveness of a **humanized, red-shifted mutant GFP by retroviral mediated gene** transfer into human tumor cells and murine fibroblasts.

#### Detailed Description Paragraph Table - DETL (2):

TABLE 2 SEQUENCE AND RESTRICTION SITE INFORMATION phGFP-S65T Humanized **GFP** Vector (SEQ ID NO:1) (GenBank Accession # U43284) Location of features Human cytomegalovirus (CMV) immediate early promoter: 152-739 Enhancer region: 229-635 TATA box: 724-730 T7 promoter: 784-803 Green fluorescent protein gene (S65T variant) Start codon (ATG): 826-828; Stop codon: 1543-1545 **GFP** fluorescent chromophore: 1021-1029 SV40 small t antigen intron: 1642-1706 SV40 early mRNA polyadenylation signal Polyadenylation signals: 2312-2317 & 2341-2346 mRNA 3' ends: 2350 & 2362 SV40 origin of replication: 2805-2740 pBR322 plasmid replication origin: 2767-3347 M13 single-strand DNA origin: 3367-3934 **Synthetic supF gene**: 4145-3947 Geneology From To 1549 811 pCDM7 vector backbone (Not I-Hind III) 1 151 Fragment from the Rous Sarcoma Virus (RSV) LTR 152 738 Fragment from Human Cytomegalovirus (CMV) containing the immediate early promoter 812 1548 **Synthetic GFP gene** using optimal human codons 817 829 Synthetic Kozak consensus translation initiation sequence 829 831 Additional valine not present in wt **GFP** 1021 1023 S65T **mutation in GFP** chromophore replacing serine 65 with threonine [Heim, R. et al. (1995) Nature 373:663-664] 1565 2174 Fragment from SV40 providing small antigen intron 2175 2415 Fragment from SV40 providing polyadenylation signals 2416 2759 Fragment from SV40 providing origin of replication 2767 3347 Fragment from pBR322 providing origin of replication 3367 3934 Fragment from M13 providing single-stranded DNA origin 3947 4145 **Synthetic supF gene** Propagation in E. coli Suitable host strain: MC1061/P3 Selectable Marker: The supF gene confers resistance to ampicillin (25-40 .mu.g/ml) and tetracycline (7.5-10 .mu.g/ml) to MC1061/P3 due to expression of a tRNA that suppresses amber **mutations** in the ampicillin and tetracycline genes on the P3 plasmid. E. coli replication origin: pBR322 (rop.sup-) Copy number: = 100-200 Plasmid incompatibility group: pMB1/ColE1

US-PAT-NO: 6518481

DOCUMENT-IDENTIFIER: US 6518481 B1

TITLE: Universal markers of transgenesis

DATE-ISSUED: February 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wimmer; Ernst A.	Bayreuth	N/A	N/A	DE
Berghammer; Andreas J.	Munchen	N/A	N/A	DE
Klingler; Martin	Munchen	N/A	N/A	DE

APPL-NO: 09/ 373129

DATE FILED: August 12, 1999

US-CL-CURRENT: 800/13, 435/320.1 , 435/455 , 435/473 , 536/24.1 , 800/21

ABSTRACT:

The invention relates to methods, cells and nucleic acids for making transgenic animals. The methods generally comprise introducing into a genome of an animal a genetic construct comprising a transcriptional regulatory element operably linked to a heterologous marker gene encoding a marker, wherein the element drives expression of the marker across genera transgenic in the construct sufficient to visually detect the marker in photoreceptive cells or organs, and selecting for transgenesis by visually detecting the marker in a photoreceptive cell or organ of the animal.

41 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (13):

To drive marker expression in a series of diverged organisms requires a promoter which is active in a wide range of species. Furthermore, to avoid problems with low expression and the interference of autofluorescence, a regional specific promoter is preferable over a constitutively active one. A wide variety of regulatory elements may be employed, so long as they meet the requisite functional limitations. These may be natural promoter elements, naturally driving gene expression in photoreceptive cells or organs, elements derived from such natural promoter elements by mutational selection or consensus sequences, synthetic elements derived by iterative selection process, e.g. SELEX procedures, etc. In a particular embodiment, the element comprises a

binding site selected from a Pax-6, a Pax-6 like binding site such as a twin-of-eyeless (TOY) binding site, a Glass binding site, etc. In more particular embodiments, the element comprises a Pax-6 Paired Domain or Homeodomain binding site, more particularly a P3 site, wherein the P3 site comprises the sequence: TAATYNRATTA (SEQ ID NO:01), wherein Y=C or T; R=G or A; N=any nucleotide (Wilson et al., 1993, Genes Dev 7, 2120-34; Czerny and Busslinger, 1995, Mol Cell Biol 15, 2858-71). Tables 1-6 provide other exemplary transcriptional regulatory element binding sites functional in the subject methods. Pax-6 binding sites are of particular interest due to the evolutionary conserved role Pax-6-homologs play in eye development across different phyla (Callaerts et al., 1997, Annu Rev Neurosci 20, 483-532).

#### Brief Summary Text - BSTX (18):

Fluorescent proteins may comprise naturally occurring, engineered (i.e., analogs) and/or synthetic sequences. For example, many cnidarians use natural green fluorescent proteins ("GFPs") as energy-transfer acceptors in bioluminescence. Natural GFPs have been isolated from numerous animals, including the Pacific Northwest jellyfish, *Aequorea victoria*, the sea pansy, *Renilla reniformis*, and *Phialidium gregarium*; Ward et al., Photochem. Photobiol., 35:803-808 (1982); Levine et al., Comp. Biochem. Physiol., 72B:77-85 (1982). In addition, a variety of *Aequorea*-related fluorescent proteins having useful excitation and emission spectra have been engineered by modifying the amino acid sequence of a naturally occurring GFP from *Aequorea victoria* (Prasher et al., Gene, 111:229-233 (1992); Heim et al., Proc. Natl. Acad. Sci., USA, 91:12501-04 (1994). Particularly useful are GFPs from or which derive from the jellyfish *A. victoria* (see e.g. U.S. Pat. No. 5,491,084 for applicable such GFPs) and include variants offering a variety of different excitation and emission wavelengths; see e.g. Heim and Tsien, 1996, Current Biology 6, 178-182. Exemplary amino acid variants include F64L, S65T, Y66W, N146I, M153T, V163A and N212K, and combinations thereof. For example, CFP is the GFP of *Aequorea victoria* with the following additional mutations: F64L, S65T, Y66W, N146I, M153T, V163A, N212K (Miyawaki et al., 1997, Nature 388:882-7), and YFP is the GFP of *A. victoria* with the following additional mutations: S65G, V68L, S72A, T203Y (Cubitt et al., 1999, Methods Cell Biol 58, 19-30). Accordingly, in preferred embodiments, the marker is a *Aequorea* or *Aequorea*-related fluorescent protein, see U.S. Pat. No. 5,912,137 for applicable sequence, scope, definitions and examples.



	L #	Hits	Search Text	DBs	Time Stamp
1	L1	32075	(humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1)	USPAT; US-PGPUB	2003/12/16 11:41
2	L2	8	1 same (regulat\$8 and codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:45
3	L3	244	(transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon adj preference	USPAT; US-PGPUB	2003/12/16 11:54
4	L4	175	3 and 1	USPAT; US-PGPUB	2003/12/16 11:55
5	L5	353	1 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:40
6	L6	3893	1 same (regulat\$8 or codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:08
7	L7	6442	1 same muta\$10	USPAT; US-PGPUB	2003/12/16 14:08
8	L8	1942	6 and 7	USPAT; US-PGPUB	2003/12/16 14:08
9	L9	52	8 and 5	USPAT; US-PGPUB	2003/12/16 14:09
10	L10	0	3 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:41
11	L11	186	3 and (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:43
12	L12	0	3 and 5	USPAT; US-PGPUB	2003/12/16 14:43
13	L13	7	(luciferase\$1 or gfp) same (codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:46

PGPUB-DOCUMENT-NUMBER: 20030157519

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030157519 A1

TITLE: Recombinant renilla reniformis system for  
bioluminescence resonance energy transfer

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Zhang, Vivian Q.	San Diego	CA	US	
Vaillancourt, Peter E.	Del Mar	CA	US	

APPL-NO: 10/ 265031

DATE FILED: October 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60329354 20011015 US

US-CL-CURRENT: 435/6, 435/191, 435/320.1, 435/325, 435/69.1, 435/7.92  
, 435/8, 536/23.2

ABSTRACT:

The invention relates to compositions comprising a first fusion protein comprising a first polypeptide domain and a R. reniformis luciferase and a second fusion protein comprising a second polypeptide domain and a R. reniformis GFP. The invention also relates to compositions comprising one or more polynucleotides encoding a first fusion protein comprising a first polypeptide domain and a R. reniformis luciferase and a second fusion protein comprising a second polypeptide domain and a R. reniformis GFP. The invention also relates to methods and kits for detecting protein-protein interactions, determining the location of a protein-protein interaction, identifying cells wherein there is a protein-protein interaction of interest, and screening for a candidate modulator that increases or decreases the amount of a protein-protein interaction.

RELATED APPLICATION(S)

[0001] This application claims the benefit of U.S. Provisional Application No. 60/329354, filed on Oct. 15, 2001. The entire teachings of the above application is incorporated herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX (70):

[0068] A polypeptide coding sequence is herein referred to as "humanized" if one or more codons is altered from the natural coding sequence to a codon which is utilized in a human but not in *Renilla*. Because there are 64 possible combinations of the 4 DNA nucleotides in codon groups of 3, the genetic code is redundant for many of the 20 amino acids. Each of the different codons for a given amino acid encodes the incorporation of that amino acid into a polypeptide. However, within a given species there tends to be a preference for certain of the redundant codons to encode a given amino acid. The "codon preference" of *R. reniformis* is different from that of humans (this codon preference is usually based upon differences in the level of expression of the tRNAs containing the corresponding anticodon sequences). In order to obtain high expression of a non-human gene product in human cells, it is advantageous to change one or more non-preferred codons to a codon sequence that is preferred in human cells. Table 1 shows the preferred codons for human gene expression. A codon sequence is preferred for human expression if it occurs to the left of a given codon sequence in the table. Optimally, but not necessarily, less preferred codons in a non-human polynucleotide coding sequence are humanized by altering them to the codon most preferred for that amino acid in human gene expression. As used herein, a GFP is "humanized" if the amount of fluorescent polypeptide expressed in a human cell from a "humanized GFP" polynucleotide sequence is at least two-fold greater, on either a mass or a fluorescence intensity scale per cell, than the amount expressed from an equal amount or number of copies of, a non-humanized GFP polynucleotide.

PGPUB-DOCUMENT-NUMBER: 20020064842

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020064842 A1

TITLE: Renilla reniformis green fluorescent protein and  
mutants thereof

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sorge, Joseph A.	Wilson	WY	US	
Vaillancourt, Peter E.	Del Mar	CA	US	
Felts, Katherine A.	San Diego	CA	US	

APPL-NO: 09/ 795040

DATE FILED: February 26, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60210561 20000609 US

US-CL-CURRENT: 435/183, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention relates to recombinant polynucleotides encoding the Green Fluorescent Protein (GFP) from *R. reniformis*, as well as polynucleotides encoding variants and fusion polypeptides of *R. reniformis* GFP. The invention further relates to vectors encoding *R. Reniformis* GFP and variants and fusions thereof, as well as to cells comprising and/or expressing such vectors. The invention also relates to recombinant *R. reniformis* GFP polypeptides and fusion polypeptides and variants thereof, as well as to methods of making and using such polypeptides both in vivo and in vitro.

----- KWIC -----

Summary of Invention Paragraph - BSTX (56):

[0054] The term "humanized polynucleotide" or "humanized sequence" refers to a polynucleotide coding sequence in which one or more, including 5 or more, 10 or more, 20 or more, 50 or more, 75 or more, 100 or more, 125 or more, 150 or more, 200 or more, or even all codons of the polynucleotide coding sequence for a non-human polypeptide (i.e., a polypeptide not naturally expressed in humans) have been altered to a codon sequence more preferred for expression in human cells. Because there are 64 possible combinations of the 4 DNA nucleotides in codon groups of 3, the genetic code is redundant for many of the 20 amino

acids. Each of the different codons for a given amino acid encodes the incorporation of that amino acid into a polypeptide. However, within a given species there tends to be a preference for certain of the redundant codons to encode a given amino acid. The "codon preference" of *R. reniformis* is different from that of humans (this codon preference is usually based upon differences in the level of expression of the tRNAs containing the corresponding anticodon sequences). In order to obtain high expression of a non-human gene product in human cells, it is advantageous to change one or more non-preferred codons to a codon sequence that is preferred in human cells. Table 1 shows the preferred codons for human gene expression. A codon sequence is preferred for human expression if it occurs to the left of a given codon sequence in the table. Optimally, but not necessarily, less preferred codons in a non-human polynucleotide coding sequence are humanized by altering them to the codon most preferred for that amino acid in human gene expression. The amount of fluorescent polypeptide expressed in a human cell from a humanized GFP polynucleotide sequence is at least two-fold greater, on either a mass or a fluorescence intensity scale per cell, than the amount expressed from an equal amount or number of copies of a non-humanized GFP polynucleotide.

US-PAT-NO: 6486382

DOCUMENT-IDENTIFIER: US 6486382 B1

TITLE: Use of the green fluorescent protein as a screenable  
marker for plant transformation

DATE-ISSUED: November 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gordan-Kamm; William	Urbandale	IA	N/A	N/A
Pierce; Dorothy A.	Urbandale	IA	N/A	N/A
Bowen; Benjamin	Des Moines	IA	N/A	N/A
Bidney; Dennis	Urbandale	IA	N/A	N/A
Ross; Margit	Johnston	IA	N/A	N/A
Scelonge; Christopher	Des Moines	IA	N/A	N/A
Miller; Michael D.	Winterset	IA	N/A	N/A
Sandahl; Gary	West Des Moines	IA	N/A	N/A
Wang; Lijuan	Urbandale	IA	N/A	N/A

APPL-NO: 09/ 214909

DATE FILED: December 20, 1999

PARENT-CASE:

This application is a United States national stage entry of PCT International Application No. PCT/US/07688, filed May 1, 1997, which claims the benefit of provisional U.S. Application No. 60/016,345, filed May 1, 1996.

PCT-DATA:

APPL-NO: PCT/US97/07688  
DATE-FILED: May 1, 1997  
PUB-NO: WO97/41228  
PUB-DATE: Nov 6, 1997  
371-DATE: Dec 20, 1999  
102(E)-DATE: Dec 20, 1999

US-CL-CURRENT: 800/278, 435/320.1, 435/419, 435/69.8, 536/23.6, 800/287, 800/298, 800/306, 800/320.1

ABSTRACT:

A method for the production of transgenic plants is provided in which a vector carrying a gene encoding the green fluorescent protein is introduced into cells, the cells are screened for the protein and transformed cells are selected and regenerated. The cellular toxicity of the green fluorescent protein is circumvented by regulating expression of the gene encoding the protein or directing the protein to a subcellular compartment where it is not

toxic to the cell. DNA constructs are provided for cell transformation in which the expression of a gene encoding the green fluorescent protein is placed under the control of an inducible promoter. In addition, DNA constructs are provided in which a nucleotide sequence encoding the green fluorescent protein is operably linked to a signal sequence which directs the expressed protein to a subcellular compartment where the protein is not toxic to the cell. Oxidative stress to plant cells transformed with GFP also can be ameliorated by transforming cells with an expression vector comprising genes encoding GFP and an oxygen scavenger enzyme such as superoxide dismutase. The toxicity of GFP in transformed plants can be eliminated by excising the screenable marker gene following detection of transformed cells or sectors. The FLP/FRT system is used in conjunction with GFP as a visible marker for transformation and FRT excision. A nucleotide sequence optimized for expression of the green fluorescent protein in plants is also provided.

33 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

----- KWIC -----

Detailed Description Text - DETX (26):

A green fluorescent protein (GFP), or a functional fragment of a GFP, is capable of producing a green fluorescence. GFP absorbs in the UV to blue range with a peak at 395 nm and emits in the green with a peak at 510 nm. The "red-shifted" version of GFP is a modified version of GFP which absorbs between 480 to 490 nm and emits in the green with a peak at 510 nm. Red-shifted GFP is abbreviated as GFP<sub>r</sub>. The "blue fluorescent protein" is a modified version of GFP which absorbs around 380 nm and emits in the blue with a peak at around 445 nm. Blue fluorescent protein is abbreviated as BFP. GFP.sub.m is a nucleotide sequence coding for GFP in which the DNA sequence has been modified based on codon preference in maize (FIG. 1).

US-PAT-NO: 6300543

DOCUMENT-IDENTIFIER: US 6300543 B1

TITLE: Transformation of zygote, egg or sperm cells and  
recovery of transformed plants from isolated embryo sacs

DATE-ISSUED: October 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cass; David D.	Edmonton	N/A	N/A	CA
McGann; Locksley E.	Spruce Grove	N/A	N/A	CA
Zhang; Guichang	Richmond Hill	N/A	N/A	CA
Laurie; John D.	Edmonton	N/A	N/A	CA
Ranch; Jerome P.	West Des Moines	IA	N/A	N/A
Gordon-Kamm; William J.	Urbandale	IA	N/A	N/A

APPL-NO: 09/ 147489

DATE FILED: May 12, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a 371 of PCT/US97/11184, filed Jul. 8, 1997, which claims the benefit of U.S. Provisional Application Ser. No. 60/021,325, filed Jul. 8, 1996, herein incorporated by reference.

PCT-DATA:

APPL-NO: PCT/US97/11184  
DATE-FILED: July 8, 1997  
PUB-NO: WO98/01576  
PUB-DATE: Jan 15, 1998  
371-DATE: May 12, 1999  
102(E)-DATE: May 12, 1999

US-CL-CURRENT: 800/278, 435/412, 435/418, 435/419, 435/424, 435/470  
, 435/69.8, 800/291, 800/300, 800/320.1

ABSTRACT:

Methodology is provided for the production of uniformly transformed plants capable of transmitting a foreign gene to progeny by sexual reproduction. A foreign gene is introduced into the zygote in an isolated embryo sac and a transformed plant is recovered. Alternatively, a foreign gene is introduced into an egg cell in an isolated embryo sac, the egg cell is fertilized with an isolated sperm cell and a transformed plant is recovered. Sperm cells may be transformed with a foreign gene, an egg cell in an isolated embryo sac is



fertilized with the transformed sperm cells, or nuclei isolated from the transformed sperm cells, and a transgenic plant is recovered. Another method for the production of transgenic plants is transformation of an embryo in an isolated embryo sac. The transgenic plant produced by any one of these methods is homogeneously transformed and capable of transmitting the foreign gene to progeny by sexual reproduction.

6 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX (29):

A green fluorescent protein (GFP), as well as a functional fragment of a GFP, is capable of producing a green fluorescence. GFP absorbs in the UV to blue range with a peak at 395 nm and emits in the green with a peak at 510 nm. GFP.sub.m is a nucleotide sequence coding for GFP in which the DNA sequence has been modified based on codon preference in maize. The nucleotide sequence of GFP.sub.m is shown in co-pending U.S. patent application Ser. No. 60/016,345, now WO97/41228, which is incorporated herein by reference.

US-PAT-NO: 6020192

DOCUMENT-IDENTIFIER: US 6020192 A

\*\*See image for Certificate of Correction\*\*

TITLE: Humanized green fluorescent protein genes and methods

DATE-ISSUED: February 1, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Muzyczka; Nicholas	Gainesville	FL	N/A	N/A
Zolotukhin; Sergei	Gainesville	FL	N/A	N/A
Hauswirth; William	Gainesville	FL	N/A	N/A

APPL-NO: 08/ 893327

DATE FILED: July 16, 1997

PARENT-CASE:

This application is a continuation-in-part of United States patent application number 08/588,201 filed Jan. 18, 1997.

US-CL-CURRENT: 435/320.1, 435/235.1 , 536/23.1 , 536/23.5

ABSTRACT:

Disclosed are synthetic and "humanized" versions of green fluorescent protein (GFP) genes adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such humanized genes are also disclosed. In addition, various methods for using the efficient expression of humanized GFP in mammalian cells and in animals are described.

11 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

----- KWIC -----

Drawing Description Text - DRTX (80):

Additional changes can also be made from considering the information in Table 3 and Table 4. These tables provide important information regarding **codon preference** in a format that is easily used. Table 3 provides a list of

the codons that are preferred for use in the humanized **gfp** constructs of the invention. Table 4 is simply the same information that incorporates U (uridine) rather than T (thymine), for ready cross-reference with FIG. 1A, FIG. 1B and FIG. 1C.

US-PAT-NO: 5968750

DOCUMENT-IDENTIFIER: US 5968750 A

TITLE: Humanized green fluorescent protein genes and methods

DATE-ISSUED: October 19, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zolotukhin; Sergei	Gainesville	FL	N/A	N/A
Muzyczka; Nicholas	Gainesville	FL	N/A	N/A
Hauswirth; William W.	Gainesville	FL	N/A	N/A

APPL-NO: 09/ 169605

DATE FILED: October 9, 1998

PARENT-CASE:

This is a divisional of application Ser. No. 08/588,201 filed Jan. 18, 1996, now U.S. Pat. No. 5,874,304.

US-CL-CURRENT: 435/6, 435/366 , 435/40.52 , 435/7.21 , 435/7.4

ABSTRACT:

Disclosed are synthetic and "humanized" versions of green fluorescent protein (GFP) genes adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such humanized genes are also disclosed. In addition, various methods for using the efficient expression of humanized GFP in mammalian cells and in animals are described.

30 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

----- KWIC -----

Detailed Description Text - DETX (65):

Additional changes can also be made from considering the information in Table 3 and Table 4. These tables provide important information regarding codon preference in a format that is easily used. Table 3 provides a list of the codons that are preferred for use in the humanized gfp constructs of the

invention. Table 4 is simply the same information that incorporates U (uridine) rather than T (thymine), for ready cross-reference with FIG. 1.

US-PAT-NO: 5874304

DOCUMENT-IDENTIFIER: US 5874304 A

TITLE: Humanized green fluorescent protein genes and methods

DATE-ISSUED: February 23, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zolotukhin; Sergei	Gainesville	FL	N/A	N/A
Muzyczka; Nicholas	Gainesville	FL	N/A	N/A
Hauswirth; William W.	Gainesville	FL	N/A	N/A

APPL-NO: 08/ 588201

DATE FILED: January 18, 1996

US-CL-CURRENT: 435/366, 435/320.1, 435/325, 435/354, 435/357, 435/358, 435/365, 435/367, 536/23.1, 536/23.5

ABSTRACT:

Disclosed are synthetic and "humanized" versions of green fluorescent protein (GFP) genes adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such humanized genes are also disclosed. In addition, various methods for using the efficient expression of humanized GFP in mammalian cells and in animals are described.

81 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

----- KWIC -----

Detailed Description Text - DETX (65):

Additional changes can also be made from considering the information in Table 3 and Table 4. These tables provide important information regarding codon preference in a format that is easily used. Table 3 provides a list of the codons that are preferred for use in the humanized gfp constructs of the invention. Table 4 is simply the same information that incorporates U (uridine) rather than T (thymine), for ready cross-reference with FIG. 1A, FIG. 1B and FIG. 1C.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	32075	(humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1)	USPAT; US-PGPUB	2003/12/16 11:41
2	L2	8	1 same (regulat\$8 and codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:45
3	L3	244	(transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon adj preference	USPAT; US-PGPUB	2003/12/16 14:50
4	L4	175	3 and 1	USPAT; US-PGPUB	2003/12/16 11:55
5	L5	353	1 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:40
6	L6	3893	1 same (regulat\$8 or codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:08
7	L7	6442	1 same muta\$10	USPAT; US-PGPUB	2003/12/16 14:08
8	L8	1942	6 and 7	USPAT; US-PGPUB	2003/12/16 14:08
9	L9	52	8 and 5	USPAT; US-PGPUB	2003/12/16 14:09
10	L10	0	3 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:50
11	L11	186	3 and (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:43
12	L12	0	3 and 5	USPAT; US-PGPUB	2003/12/16 14:43
13	L13	7	(luciferase\$1 or gfp) same (codon adj preference)	USPAT; US-PGPUB	2003/12/16 14:46
14	L14	12712	(transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon	USPAT; US-PGPUB	2003/12/16 14:50
15	L15	384	14 same (luciferase\$1 or gfp)	USPAT; US-PGPUB	2003/12/16 14:51
16	L16	7469	14 and 1	USPAT; US-PGPUB	2003/12/16 14:51
17	L17	192	15 and 1	USPAT; US-PGPUB	2003/12/16 14:52
18	L18	78	15 and 7	USPAT; US-PGPUB	2003/12/16 14:52

PGPUB-DOCUMENT-NUMBER: 20030224444

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030224444 A1

TITLE: Antibodies to native conformations of membrane proteins

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Berkley, Neil	San Diego	CA	US	
Surber, Mark W.	Coronado	CA	US	

APPL-NO: 10/ 157491

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/7.1, 435/326 , 435/69.1 , 530/387.1

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

[0005] All of the preceding applications are hereby incorporated in their entirety (including drawings) by reference thereto.

----- KWIC -----



Detail Description Paragraph - DETX (730):

[0888] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (854):

[1000] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030224369

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030224369 A1

TITLE: Reverse screening and target identification with  
minicells

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Surber, Mark W.	Coronado	CA	US	
Berkley, Neil	San Diego	CA	US	
Gerhart, William	La Mesa	CA	US	

APPL-NO: 10/ 157171

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/6

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (726):

[0904] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (850):

[1009] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030219888

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030219888 A1

TITLE: Minicell-based bioremediation

PUBLICATION-DATE: November 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Segall, Anca M.	San Diego	CA	US	
Klepper, Robert	San Diego	CA	US	

APPL-NO: 10/ 157418

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157418 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 435/262.5

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

[0005] All of the preceding applications are hereby incorporated in their entirety (including drawings) by reference thereto.

----- KWIC -----

Detail Description Paragraph - DETX (821):

[0930] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (946):

[1034] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO: 42, and the downstream primer had the sequence SEQ ID NO: 43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030219408

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030219408 A1

TITLE: Methods of making pharmaceutical compositions with  
minicells

PUBLICATION-DATE: November 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Klepper, Robert	San Diego	CA	US	

APPL-NO: 10/ 157320

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157320 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 424/93.2

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (818):

[0994] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (942):

[1099] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030217377

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030217377 A1

TITLE: Targeted chromosomal genomic alterations with modified  
single stranded oligonucleotides

PUBLICATION-DATE: November 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kmiec, Eric B.	Landenberg	PA	US	
Gamper, Howard B.	Philadelphia	PA	US	
Rice, Michael C.	Newtown	PA	US	

APPL-NO: 10/ 209787

DATE FILED: July 30, 2002

RELATED-US-APPL-DATA:

child 10209787 A1 20020730

parent division-of 09818875 20010327 US PENDING

non-provisional-of-provisional 60244989 20001030 US

non-provisional-of-provisional 60208538 20000601 US

non-provisional-of-provisional 60192176 20000327 US

non-provisional-of-provisional 60192179 20000327 US

US-CL-CURRENT: 800/8, 435/325 , 435/455 , 435/6 , 514/44 , 536/23.2

ABSTRACT:

Presented are methods and compositions for targeted chromosomal genomic alterations using modified single-stranded oligonucleotides. The oligonucleotides of the invention have at least one modified nuclease-resistant terminal region comprising phosphorothioate linkages, LNA analogs or 2'-O-Me base analogs.

----- KWIC -----

Brief Description of Drawings Paragraph - DRTX (8):

[0046] FIG. 7. Hygromycin-eGFP target plasmids. (A) Plasmid



pAURHYG(ins)**GFP** contains a single base insertion mutation between nucleotides 136 and 137, at **codon 46**, of the Hygromycin B coding sequence (cds) which is transcribed from the constitutive ADH1 **promoter**. The target sequence presented below indicates the deletion of an A and the substitution of a C for a T directed by the oligonucleotides to re-establish the resistant phenotype. (B) Plasmid pAURHYG(rep)**GFP** contains a base substitution mutation introducing a G at nucleotide 137, at **codon 46**, of the Hygromycin B coding sequence (cds). The target sequence presented below the diagram indicates the amino acid conservative replacement of G with C, restoring gene function.

#### Detail Description Paragraph - DETX (4):

[0051] In this example, single-stranded and double-hairpin oligonucleotides with chimeric backbones (see FIG. 1 for structures (A and B) and sequences (C and D) of assay oligonucleotides) are used to correct a point **mutation** in the kanamycin gene of pK.sup.sm4021 (FIG. 2) or the tetracycline gene of pT.sup.s.DELTA.208 (FIG. 3). All kan oligonucleotides share the same 25 base sequence surrounding the target base identified for change, just as all tet oligonucleotides do. The sequence is given in FIGS. 1C and FIG. 1D. Each plasmid contains a functional ampicillin gene. Kanamycin gene function is restored when a G at position 4021 is converted to a C (via a substitution **mutation**); tetracycline gene function is restored when a deletion at position 208 is replaced by a C (via frameshift **mutation**). A separate plasmid, pAURNeo(-)FIAsH (FIG. 9), bearing the kan.sup.s gene is used in the cell culture experiments. This plasmid was constructed by inserting a **synthetic expression cassette containing a neomycin phosphotransferase (kanamycin resistance) gene** and an extended reading frame that encodes a receptor for the FIAsH ligand into the pAUR123 shuttle vector (Panvera Corp., Madison, Wis.). The resulting construct replicates in *S. cerevisiae* at low copy number, confers resistance to aureobasidinA and constitutively expresses either the Neo+/FIAsH fusion product (after alteration) or the truncated Neo-/FIAsH product (before alteration) from the ADH1 promoter. By extending the reading frame of this gene to code for a unique peptide sequence capable of binding a small ligand to form a fluorescent complex, restoration of expression by correction of the stop codon can be detected in real time using confocal microscopy. Additional constructs can be made to test additional gene alteration events.

PGPUB-DOCUMENT-NUMBER: 20030211599

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030211599 A1

TITLE: Minicell-based delivery agents

PUBLICATION-DATE: November 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Klepper, Robert	San Diego	CA	US	
Surber, Mark W.	Coronado	CA	US	

APPL-NO: 10/ 157106

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157106 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 435/325, 435/252.3

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (818):

[0994] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (942):

[1099] A **GFP** fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030211086

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030211086 A1

TITLE: Minicell-based selective absorption

PUBLICATION-DATE: November 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Berkley, Neil	San Diego	CA	US	
Sabbadini, Roger A.	Lakeside	CA	US	

APPL-NO: 10/ 157073

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295566 20010605 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 424/93.21, 424/1.49 , 424/1.73 , 435/325

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Ser. No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Ser. No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Serial No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

[0005] All of the preceding applications are hereby incorporated in their entirety (including drawings) by reference thereto.

----- KWIC -----

Detail Description Paragraph - DETX (728):

[0906] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (895):

[1063] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030207833

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030207833 A1

TITLE: Pharmaceutical compositions with minicells

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Berkley, Neil	San Diego	CA	US	
Klepper, Robert	San Diego	CA	US	
Sabbadini, Roger A.	Lakeside	CA	US	

APPL-NO: 10/ 156811

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 514/44, 424/93.21

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (782):

[0959] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (909):

[1067] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030203481

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030203481 A1

TITLE: Conjugated minicells

PUBLICATION-DATE: October 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Surber, Mark W.	Coronado	CA	US	
Klepper, Robert	San Diego	CA	US	

APPL-NO: 10/ 157213

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/325

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (726):

[0904] Polypeptides having deletions or insertions of naturally occurring



amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (850):

[1009] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030203411

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030203411 A1

TITLE: Methods of minicell-based delivery

PUBLICATION-DATE: October 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Berkley, Neil	San Diego	CA	US	
Klepper, Robert	San Diego	CA	US	
Surber, Mark W.	Coronado	CA	US	

APPL-NO: 10/ 156792

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295566 20010605 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/7.2, 424/1.49

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (818):

[0994] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (942):

[1099] A **GFP** fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the **GFP** into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the **GFP** protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding **GFP** operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030202937

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030202937 A1

TITLE: Minicell-based diagnostics

PUBLICATION-DATE: October 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Klepper, Robert	San Diego	CA	US	
Berkley, Neil	San Diego	CA	US	

APPL-NO: 10/ 157178

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295566 20010605 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 424/1.49, 424/9.34 , 424/9.5

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Serial No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

[0005] All of the preceding applications are hereby incorporated in their entirety (including drawings) by reference thereto.

----- KWIC -----

Detail Description Paragraph - DETX (730):

[0909] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (897):

[1057] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030199089

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030199089 A1

TITLE: Membrane to membrane delivery

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Surber, Mark W.	Coronado	CA	US	
Sabbadini, Roger A.	Lakeside	CA	US	

APPL-NO: 10/ 157318

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295566 20010605 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/449, 435/455

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (818):

[0994] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (953):

[1110] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030199088

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030199088 A1

TITLE: Minicell-based gene therapy

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Berkley, Neil	San Diego	CA	US	
Surber, Mark W.	Coronado	CA	US	

APPL-NO: 10/ 156902

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295566 20010605 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/449, 435/320.1 , 435/325

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----



Detail Description Paragraph - DETX (817):

[0993] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (841):

[1098] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030199005

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030199005 A1

TITLE: Solid supports with minicells

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger	Lakeside	CA	US	
Klepper, Robert	San Diego	CA	US	

APPL-NO: 10/ 157166

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157166 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 435/7.21, 435/325

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (728):

[0906] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (852):

[1011] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030198996

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030198996 A1

TITLE: Minicell libraries

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Surber, Mark W.	Coronado	CA	US	
Berkley, Neil	San Diego	CA	US	
Gerhart, William	La Mesa	CA	US	
Sabbadini, Roger A.	Lakeside	CA	US	

APPL-NO: 10/ 157147

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157147 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60293566 20010524 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/7.1, 435/325

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Serial No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of

Making Minicells," by Surber, et al., filed May 24, 2002.

[0005] All of the preceding applications are hereby incorporated in their entirety (including drawings) by reference thereto.

----- KWIC -----

Detail Description Paragraph - DETX (785):

[0964] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (952):

[1121] A **GFP** fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the **GFP** into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the **GFP** protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding **GFP** operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030198995

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030198995 A1

TITLE: Forward screening with minicells

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Berkley, Neil	San Diego	CA	US	
Surber, Mark W.	Coronado	CA	US	
Gerhart, William	La Mesa	CA	US	

APPL-NO: 10/ 156831

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10156831 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 435/7.1, 435/5, 435/7.21

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Ser. No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Ser. No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. (attorney docket No. 089608-0401), entitled "Methods of Making

Minicells," by Surber, et al., filed May 24, 2002.

[0005] All of the preceding applications are hereby incorporated in their entirety (including drawings) by reference thereto.

----- KWIC -----

Detail Description Paragraph - DETX (730):

[0909] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (854):

[1014] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030194798

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030194798 A1

TITLE: Minicell compositions and methods

PUBLICATION-DATE: October 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Surber, Mark W.	Coronado	CA	US	
Sabbadini, Roger A.	Lakeside	CA	US	

APPL-NO: 10/ 154951

DATE FILED: May 24, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60293566 20010524 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/252.1, 435/252.3

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

----- KWIC -----

Brief Description of Drawings Paragraph - DRTX (825):

[0998] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (46):

[1103] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy



used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030194714

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030194714 A1

TITLE: Minicell-based transformation

PUBLICATION-DATE: October 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Berkley, Neil	San Diego	CA	US	
Surber, Mark W.	Coronado	CA	US	

APPL-NO: 10/ 157299

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295566 20010605 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/6, 435/325 , 435/455

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Ser. No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Ser. No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Serial No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

[0005] All of the preceding applications are hereby incorporated in their entirety (including drawings) by reference thereto.

----- KWIC -----

Detail Description Paragraph - DETX (819):

[0997] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (946):

[1114] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030190749

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190749 A1

TITLE: Minicell-producing parent cells

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Surber, Mark W.	Coronado	CA	US	
Sabbadini, Roger A.	Lakeside	CA	US	
Segall, Anca M.	San Diego	CA	US	
Berkley, Neil	San Diego	CA	US	

APPL-NO: 10/ 157215

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157215 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 435/375

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Ser. No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Ser. No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of

----- KWIC -----

Detail Description Paragraph - DETX (822):

[0975] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (948):

[1078] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030190683

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190683 A1

TITLE: Minicell-based rational drug design

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Surber, Mark W.	Coronado	CA	US	

APPL-NO: 10/ 157302

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157302 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 435/7.21, 435/325 , 702/19

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (818):

[0994] Polypeptides having deletions or insertions of naturally occurring amino acids may be **synthetic oligopeptides that result from the chemical synthesis of amino acid sequences** that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed **mutagenesis**.

Detail Description Paragraph - DETX (945):

[1102] A **GFP** fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the **GFP** into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop **codon**; this allowed for the in frame incorporation of the CBP fusion tag to the **GFP** protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding **GFP** operably linked to a T7 **promoter** and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030190601

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190601 A1

TITLE: Target display on minicells

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Berkley, Neil	San Diego	CA	US	
Surber, Mark W.	Coronada	CA	US	

APPL-NO: 10/ 157096

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157096 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 435/5, 435/6 , 435/7.1 , 435/7.21

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Serial No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.



[0005] All of the preceding applications are hereby incorporated in their entirety (including drawings) by reference thereto.

----- KWIC -----

Detail Description Paragraph - DETX (824):

[0983] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (952):

[1090] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030186356

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030186356 A1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Stemmer, Willem P.C.	Los Gatos	CA	US	

APPL-NO: 10/ 246229

DATE FILED: September 17, 2002

RELATED-US-APPL-DATA:

child 10246229 A1 20020917

parent continuation-of 09717391 20001118 US GRANTED

parent-patent 6506603 US

child 09717391 20001118 US

parent continuation-of 09239395 19990128 US ABANDONED

child 09239395 19990128 US

parent continuation-of 08621859 19960325 US GRANTED

parent-patent 6117679 US

child 08621859 19960325 US

parent continuation-in-part-of 08564955 19951130 US GRANTED

parent-patent 5811238 US

child 08564955 19951130 US

parent continuation-in-part-of PCT/US95/02126 19950217 US PENDING

child PCT/US95/02126 19950217 US

parent continuation-in-part-of 08198431 19940217 US GRANTED

parent-patent 5605793 US

US-CL-CURRENT: 435/68.1, 435/69.1, 536/23.1

#### ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

----- KWIC -----

#### Detail Description Paragraph - DETX (90):

[0194] The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

#### Detail Description Paragraph - DETX (97):

[0201] By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detail Description Paragraph - DETX (102):

[0206] This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detail Description Paragraph - DETX (168):

[0272] Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14:256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269:9533). Riechmann et al. (1993) Biochemistry 32:8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

Detail Description Paragraph - DETX (174):

[0278] For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detail Description Paragraph - DETX (489):

[0575] A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR

and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

Detail Description Paragraph - DETX (505):

[0587] E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and GFP promoters are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

PGPUB-DOCUMENT-NUMBER: 20030182672

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030182672 A1

TITLE: Genetic silencing

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Graham, Michael Wayne	Chapel Hill		AU	
Rice, Robert Norman	Sinnamon Park		AU	
Reed, Kenneth Clifford	St. Lucia		AU	
Murphy, Kathleen Margaret	Rocklea		AU	

APPL-NO: 10/ 245805

DATE FILED: September 16, 2002

RELATED-US-APPL-DATA:

child 10245805 A1 20020916

parent continuation-of PCT/AU01/00297 20010316 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	01/70949	2001WO-01/70949	September 27, 2001
AU	PQ 6363	2000AU-PQ 6363	March 17, 2000
AU	PR 2700	2001AU-PR 2700	January 24, 2001

US-CL-CURRENT: 800/18, 435/320.1 , 435/325 , 435/372 , 435/455 , 435/69.1

ABSTRACT:

A method of inducing, promoting or otherwise facilitating a change in the phenotype of an animal cell or group of animal cells including an animal comprising said cells. The modulation of phenotypic expression is conveniently accomplished via genotypic manipulation through such means as reducing translation of transcript to proteinaceous product. The ability to induce, promote or otherwise facilitate the silencing of expressible genetic sequences provides a means for modulating the phenotype in, for example, the medical, veterinary and the animal husbandry industries. Expressible genetic sequences contemplated by the present invention include not only genes normally resident in a particular animal cell (i.e., indigenous genes) but also genes introduced through recombinant means or through infection by pathogenic agents such as viruses.

Detail Description Paragraph - DETX (27):

[0082] Generally, a synthetic gene of the instant invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions without affecting its ability to modify target gene expression. Nucleotide insertional derivatives of the synthetic gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity.

Detail Description Paragraph - DETX (257):

[0301] Plasmid pEGFP-N1 (FIG. 1; Clontech) contains the CMV IE promoter operably connected to an open reading frame encoding a red-shifted variant of the wild-type GFP which has been optimized for brighter fluorescence. The specific GFP variant encoded by pEGFP-N1 has been disclosed by Cormack et al. (1996). Plasmid pEGFP-N1 contains a multiple cloning site comprising BglII and BamHI sites and many other restriction endonuclease cleavage sites, located between the CMV IE promoter and the EGFP open reading frame. The plasmid pEGFP-N1 will express the EGFP protein in mammalian cells. In addition, structural genes cloned into the multiple cloning site will be expressed as EGFP fusion polypeptides if they are in-frame with the EGFP-encoding sequence and lack a functional translation stop codon. The plasmid further comprises an SV40 polyadenylation signal downstream of the EGFP open reading frame to direct proper processing of the 3'-end of mRNA transcribed from the CMV IE promoter sequence (SV40 pA). The plasmid further comprises the SV40 origin of replication functional in animal cells; the neomycin-resistance gene comprising SV40 early promoter (SV40-E in FIG. 1) operably connected to the neomycin/kanamycin-resistance gene derived from Tn5 Kan/Neo in FIG. 1) and the HSV thymidine kinase polyadenylation signal, for selection of transformed cells on kanamycin, neomycin or geneticin; the pUC19 origin of replication which is functional in bacterial cells and the f1 origin of replication for single-stranded DNA production.

PGPUB-DOCUMENT-NUMBER: 20030166279

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030166279 A1

TITLE: Minicell-based transfection

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Berkley, Neil	San Diego	CA	US	

APPL-NO: 10/ 157391

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

child 10157391 A1 20020528

parent division-of 10154951 20020524 US PENDING

non-provisional-of-provisional 60359843 20020225 US

non-provisional-of-provisional 60293566 20010524 US

US-CL-CURRENT: 435/449, 435/320.1 , 435/325

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.



----- KWIC -----

Detail Description Paragraph - DETX (728):

[0906] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (852):

[1011] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030166099

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030166099 A1

TITLE: Minicells comprising membrane proteins

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	
Surber, Mark W.	Coronado	CA	US	
Berkley, Neil	San Diego	CA	US	
Segall, Anca M.	San Diego	CA	US	
Klepper, Robert	San Diego	CA	US	

APPL-NO: 10/ 157305

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295566 20010605 US

non-provisional-of-provisional 60359843 20020225 US

US-CL-CURRENT: 435/69.1, 435/325

ABSTRACT:

The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

RELATED APPLICATIONS

[0001] This application claims priority to the following U.S. patent applications:

[0002] Serial No. 60/295,566 entitled "Minicell Compositions and Methods" by Roger Sabbadini, filed May 24, 2001;

[0003] Serial No. 60/359,843 entitled "Minicell Compositions and Methods" by Sabbadini, et al., filed Feb. 25, 2002; and

[0004] Ser. No. \_\_\_\_\_ (attorney docket No. 089608-0401), entitled "Methods of Making Minicells," by Surber, et al., filed May 24, 2002.

----- KWIC -----

Detail Description Paragraph - DETX (817):

[0993] Polypeptides having deletions or insertions of naturally occurring amino acids may be synthetic oligopeptides that result from the chemical synthesis of amino acid sequences that are based on the amino acid sequence of a parent polypeptide but which have one or more amino acids inserted or deleted relative to the sequence of the parent polypeptide. Insertions and deletions of amino acid residues in polypeptides having longer amino acid sequences may be prepared by directed mutagenesis.

Detail Description Paragraph - DETX (984):

[1141] A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

PGPUB-DOCUMENT-NUMBER: 20030165873

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030165873 A1

TITLE: Three hybrid assay system

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Come, Jon H.	Cambridge	MA	US	
Becker, Frank	Planegg	MA	DE	
Kley, Nikolai	Wellesley		US	

APPL-NO: 10/ 091177

DATE FILED: March 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60272932 20010302 US

non-provisional-of-provisional 60278233 20010323 US

non-provisional-of-provisional 60329437 20011015 US

US-CL-CURRENT: 435/6, 435/7.1, 530/350, 536/23.1, 536/5, 552/570

ABSTRACT:

The invention provides compositions and methods for isolating ligand binding polypeptides for a user-specified ligand, and for isolating small molecule ligands for a user-specified target polypeptide using an improved class of hybrid ligand compounds.

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional application No. 60/272,932, filed on Mar. 2, 2001; U.S. Provisional application No. 60/278,233, filed on Mar. 23, 2001; and U.S. Provisional application No. 60/329,437, filed on Oct. 15, 2001, the specifications of which are hereby incorporated by reference in their entirety.

----- KWIC -----

Detail Description Paragraph - DETX (101):

[0188] The reporter moiety may be a transcription factor tethered to a

cellular membrane preventing entry into the nucleus and transcriptional activation. Only upon re-association of the ubiquitin halves after compound-protein interaction, the reporter moiety will be released and translocate into the nucleus where transcription of a reporter gene may be activated. Reporter genes may be enzymes, fluorescent markers or nutritional markers (e.g. lacZ, green fluorescent protein **GFP**/yeast **codon** optimized red fluorescent protein yRFP, HIS/URA) (Stagljär et al. (1998) Proc. Natl. Acad. Sci. U.S.A., 95: 5187-92).

Detail Description Paragraph - DETX (313):

[0400] In one embodiment, the variegated library of variants is generated by combinatorial **mutagenesis** at the nucleic acid level, and is encoded by a variegated gene library. For instance, a mixture of **synthetic oligonucleotides** **can be enzymatically ligated into gene** sequences such that the degenerate set of potential sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of sequences therein.

PGPUB-DOCUMENT-NUMBER: 20030159161

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030159161 A1

TITLE: Synthetic genes and genetic constructs comprising same  
I

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Graham, Michael Wayne	Jindalee		AU	
Rice, Robert Norman	Sinnamon Park		AU	

APPL-NO: 10/ 346853

DATE FILED: January 17, 2003

RELATED-US-APPL-DATA:

child 10346853 A1 20030117

parent continuation-of 09100812 19980619 US GRANTED

parent-patent 6573099 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
AU	PP2292	1998AU-PP2292	March 20, 1998

US-CL-CURRENT: 800/8, 435/320.1 , 435/325 , 435/455

ABSTRACT:

The present invention relates generally to synthetic genes for modifying endogenous gene expression in a cell, tissue or organ of a transgenic organism, in particular a transgenic animal or plant. More particularly, the present invention provides novel synthetic genes and genetic constructs which are capable of repressing delaying or otherwise reducing the expression of an endogenous gene or a target gene in an organism when introduced thereto.

----- KWIC -----

Detail Description Paragraph - DETX (16):

[0057] Generally, a gene of the invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions without affecting its ability to modify target gene expression. Nucleotide

insertional derivatives of the synthetic gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product.

Detail Description Paragraph - DETX (26):

[0067] Alternatively, the structural gene may comprise a nucleotide sequence which does not encode an amino acid sequence or more commonly, comprises one or more open reading frames which encode one or more peptides, oligopeptides or polypeptides which are unrelated at the amino acid sequence level to the amino acid sequence encoded by the target gene. For example, the mRNA product of the structural gene may be inserted into the synthetic gene of the invention so as to alter or disrupt the reading frame of the structural gene and produce one or more frame shift mutations in the translation product thereof relative to the translation product encoded by the target gene, notwithstanding a substantial identity between the structural gene and the target gene on the one hand and the corresponding mRNA products of the structural gene and the target gene on the other hand. Such effects may be produced by introducing one or two nucleotide substitutions or deletions into the structural gene, relative to the target gene sequence or alternatively, by introducing a translation start codon 5'-ATG-3' upstream of any nucleotide in the structural gene which occurs at a particular position in a codon of the corresponding target gene such that the position of said nucleotide in the codon of the structural gene is altered.

Detail Description Paragraph - DETX (179):

[0204] Plasmid pCMVLacI.TYR.OPRSV1.GFP (FIG. 28) is a dual construct in which the CMV IE promoter drives expression of the lacI gene and the mRNA of the mouse tyrosinase cDNA or a fragment thereof, whilst the OPRSVI promoter drives expression of GFP operably under control of the lacI gene. The construct is designed such that the mouse tyrosinase gene is fused to the 3' untranslated region of the lacI gene via a unique BsaB1 cloning site. This cloning site is located after the stop codon of the lacI coding sequence, but before the SV40 polyadenylation signal. The construct also contains the hygromycin-resistance gene as a selection marker.

PGPUB-DOCUMENT-NUMBER: 20030154500

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030154500 A1

TITLE: Nucleic acid transfer vector for the introduction of  
nucleic acid into the DNA of a cell

PUBLICATION-DATE: August 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hackett, Perry B.	Shoreview	MN	US	
Clark, Karl J.	Ramsey	MN	US	
Ivics, Zoltan	Berlin	MN	DE	
Izsvak, Zsuzsanna	Berlin		DE	
Fahrenkrug, Scott C.	St. Paul		US	

APPL-NO: 10/ 191698

DATE FILED: July 9, 2002

RELATED-US-APPL-DATA:

child 10191698 A1 20020709

parent continuation-of 09191572 19981113 US ABANDONED

US-CL-CURRENT: 800/8, 435/320.1 , 435/325 , 435/69.1 , 530/350 , 536/23.5

ABSTRACT:

The present invention provides for transposon vectors encoding expression control region-traps and gene-traps. Also provided are dicistronic vectors. Certain embodiments of the invention contain internal ribosome entry sites.

CONTINUING APPLICATION DATA

[0001] This application is a continuation application of U.S. Ser. No. 09/191,572, which is incorporated by reference in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX (103):

[0146] Accordingly, a series of constructs was made to bring the coding sequence closer, step-by-step, to the consensus using PCR mutagenesis (SB4 through SB10 in FIG. 1B). As a general approach the sequence information predicted by the majority-rule consensus was followed. However, at some codons



deamination of .sup.5mC residues of CpG sites occurred, and C->T mutations had been fixed in many elements. At R(288), where TpG's and CpG's were represented in equal numbers in the alignment, the CpG sequence was chosen because the CpG->TpG transition is more common in vertebrates than the TpG->CpG. The result of this extensive genetic engineering is a synthetic transposase gene encoding 340 amino acids (SB10 in FIGS. 1B and 2).

Detail Description Paragraph - DETX (167):

[0193] pnBeG: pnBeG was constructed of component fragments of SK/nBeG(afmx) and pBL. Both vectors were digested with the restriction endonucleases SacI and XmaI. SacI cuts within the amp resistance gene and XmaI cuts just upstream of the .beta.-galactosidase gene in either vector. The 6.67-kb XmaI/SacI fragment of SK/nBeG(afmx) was ligated to the 1.35-kb SacI/XmaI fragment of pBL. This regenerated the amp resistance gene and replaced the T7 promoter region of SK/nBeG(afmx) with the CMV/T7 promoters located within pBL. pnBeG was further optimized by PCR mutagenesis of the IRES-GFP junction to GAAAAACACGATTGCTATATGGCCACA ACCATGGCTAGC (SEQ ID NO:64). This sequence restored wild-type EMCV IRES spacing from the polypyrimidine tract to the ATG start codon (double underline), as well as restoring the wild-type sequence around the start codon. Incorporation of an NheI restriction endonuclease site (italics) allowed a fusion with the unique NheI restriction site in the Affymax GFP (Affymax, Santa Clara, Calif.). One fusion site is four amino acids downstream of the EMCV IRES initiation codon. The fusion also recreated the NcoI restriction endonuclease site (underlined), which is found in some strains of EMCV. The Affymax GFP is a GFP that has been modified to fluoresce more than GFP. Also, the 0.56-kb MscI/EcoRI fragment of pXex-GM2 (Obtained from Shao Lin, Dept. of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, Ga.) was used to replace the 0.55-kb MscI/EcoRI fragment of pnBeG. This moved the chromophore and C-terminus of an enhanced GFP (GM2) (B. P. Cormack et al., Gene, 173(1 Spec No), 33-8 (1996)) into pnBeG. GM2 is a GFP that has been modified to fluoresce more than Affymax GFP. The construct with the optimal spacing between the EMCV IRES and the GM2 was named pnBeG\*.

Detail Description Paragraph - DETX (186):

[0210] pFV/e(nls)G: pFV/e(nls)G was formed from components of pFV3 (Caldovic L., et al., Mol. Mar. Biol. Biotechnol., 4, 51-61 (1995)) and pnBeG\*. pFV3 was first digested by EcoRI. This linearized pFV3 just 3' of the CSGH poly(A) signal. After digestion with EcoRI, the recessed ends of pFV3 were completely filled in using Klenow polymerase. The resultant fragment was self-ligated to form pFV3.DELTA.RI. A double-stranded oligo, FV7-MCS (CGGGGTACCGAATTCCCGGGTACCCCG) (SEQ ID NO:66) containing an EcoRI and SmaI sites within KpnI sites, was digested with KpnI. This oligo was then cloned into pFV3.DELTA.RI cut with KpnI, which cuts once just after the carp .beta.-actin intron 1. There were two products of this ligation, pFV7a and pFV7b. pFV7a has the SmaI site preceding the EcoRI site in relationship to the carp .beta.-actin promoter, carp .beta.-actin exon 1, carp .beta.-actin intron 1, and the CSGH poly(A) signal. pnBeG\* was then cut with EcoRI. One of the resulting fragments of this digest contained only the EMCV IRES and GFP. This fragment was then cloned into pFV7a digested with EcoRI. The product that

contained the EMCV IRES and **GFP** in the proper orientation with respect to the fish elements (i.e. **promoter**, exon, intron, poly(A) signal) was named pFV/eG. pFV/eG was then digested with the restriction endonuclease NheI that cuts just after the initiation **codon of GFP**. Into this site a short double-stranded oligo, NLS2 (TACTCCACCAAGAAGAGAAAGGT GGAGGACG (SEQ ID NO:67) with CTAG 5' end overhangs), was ligated. One of the resulting products of this ligation, pFV/e(nls)G, has an additional 12 amino acids (TPPKRKVE DAS) (SEQ ID NO:68) encoding the SV40 nuclear localization signal.

PGPUB-DOCUMENT-NUMBER: 20030149995

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030149995 A1

TITLE: Compound screening methods

PUBLICATION-DATE: August 7, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 371101

DATE FILED: February 21, 2003

RELATED-US-APPL-DATA:

child 10371101 A1 20030221

parent division-of 09549872 20000414 US GRANTED

parent-patent 6540996 US

non-provisional-of-provisional 60129596 19990415 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	GB 9908670.4	1999GB-GB 9908670.4	April 15, 1999
GB	GB 9912736.7	1999GB-GB 9912736.7	June 1, 1999

US-CL-CURRENT: 800/3, 800/8

ABSTRACT:

The invention provides methods of screening for compounds which affect the activity of a physiologically important calcium pump, the sarco/endoplasmic reticulum Ca.sup.2+ ATPase (SERCA), using the nematode worm *C. elegans*.

RELATED APPLICATIONS

[0001] This application is a divisional of application Ser. No. 09/549,872 filed Apr. 14, 2000, now pending. This application claims priority under Title 35 U.S.C. .sctn.119(e), of U.S. provisional application No. 60/129,596, filed Apr. 15, 1999, entitled COMPOUND SCREENING METHODS, and foreign priority benefits under Title 35, U.S.C., .sctn.119(a)-(d) or .sctn.365(a),(b) of foreign patent application. Nos. GB 9908670.4, filed Apr. 15, 1999, & GB

9912736.7, filed Jun. 1, 1999, the entire contents of which are incorporated herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX (16):

[0137] A 5026 bp fragment of the upstream region of the *C. elegans* SERCA gene, starting 5026 bp upstream of the translation initiation codon and continuing up to and including the A of the ATG initiation codon (SEQ ID NO:3), was cloned into the vector pPD95.79 (described in Fire et al. (1990) Gene, 93: 189-198) in fusion with a GFP fluorescent protein, resulting in vector pGK10. The cloned fragment can be considered as the promoter region of the *C. elegans* SERCA. The vector was injected into *C. elegans*, using standard methodology well known to persons skilled in the art, and the expression of the GFP was monitored applying standard fluorescent techniques. GFP expression was observed all over the early embryo of the worm, although expression was faint in some tissues. In a later stage of development, from mid-embryo stage, through larval stage to adult stage, strong GFP expression could be observed in all muscle tissue, including the pharyngeal muscles, the body wall muscles, the anal depressor and the anal sphincter. In adults staining was seen in the vulva muscles, the uterine muscles, the spermathecae and the proximal myoepithelial sheath cells of the gonad.

Detail Description Paragraph - DETX (17):

[0138] A construct containing a smaller promoter fragment, including A of the initiating ATG codon and extending 2915 bp upstream (SEQ ID NO:4), fused to a GFP gene was generated by a PstI deletion of the plasmid pGK10. This plasmid was designated pGK13. Transfection of the nematode with pGK13 resulted in the same pattern of GFP expression as was observed with pGK10.

Detail Description Paragraph - DETX (116):

[0228] oGK52 contains T-to-G point mutation compared to pig PLB cDNA so as to introduce a D-to-E amino acid substitution at position 2 of PLB. Since this is the only difference between the human and pig PLB proteins, the resultant polypeptide is the same as the human PLB sequence (NB the point mutated cDNA does not have the same sequence as the human PLB cDNA but encodes a protein having identical amino acid sequence to human PLB, hence it is referred to as a humanized pig PLB cDNA). Sequences of the primers are as follows:

PGPUB-DOCUMENT-NUMBER: 20030149254

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030149254 A1

TITLE: Methods and compositions comprising Renilla GFP

PUBLICATION-DATE: August 7, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 133973

DATE FILED: April 24, 2002

RELATED-US-APPL-DATA:

child 10133973 A1 20020424

parent continuation-of 09710058 20001110 US PENDING

non-provisional-of-provisional 60290287 20010510 US

non-provisional-of-provisional 60164592 19991110 US

US-CL-CURRENT: 536/23.1, 435/320.1 , 435/325 , 435/6 , 435/69.7 , 530/350

ABSTRACT:

The invention relates to methods and compositions utilizing Renilla green fluorescent proteins (rGFP), and Ptilosarcus green fluorescent proteins (pGFP). In particular, the invention relates to the use of Renilla GFP or Ptilosarcus GFP proteins as reporters for cell assays, particularly intracellular assays, including methods of screening libraries using rGFP or pGFP.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of the filing date of application U.S. Serial No. 60/290,287 filed May 10, 2001 and of application U.S. Ser. No. 09/710,058, filed Nov. 10, 2000.

----- KWIC -----

Detail Description Paragraph - DETX (56):

[0078] The codon optimized GFPs are made in accordance with methods well

known in the art. When the substitutions or replacements are not extensive, oligonucleotide directed mutagenesis or other localized mutagenesis techniques, such as replacing fragments of the gene with fragments containing the preferred codons, are used to optimize the codons. If codon optimization is extensive, the GFP gene may be a synthetic gene generated from overlapping oligonucleotides (Jayaraman, K. et al. (1991) Proc. Natl. Acad. Sci. USA 88: 4084-8; Stemmer, W. P. et al. (1995) Gene 164: 49-53). The oligonucleotides may or may not be ligated together during the process for generating the synthetic gene. In this regard, use of polymerase chain reaction of the hybridized overlapping oligonucleotides allows facile generation of these synthetic genes.

Detail Description Paragraph - DETX (352):

[0371] Retroviral constructs were based on a pCGFP vector that carries a composite CMV promoter fused to the transcriptional start site of the MMLV R-U5 region of the LTR, and extended packaging sequence, deletion of the MMLV gag start ATG, and a multiple cloning region encoding EGFP, an Aequoria Victoria GFP variant codon optimized for expression in human cells (Clontech, Palo Alto, Calif.) and a Kozak consensus start, described in Kozak (1986) Cell 44: 283-292. The vector used to express flag tagged EGFP, pEf, is identical to pCGFP but has additional restriction sites in the open reading frame of EGFP (resulting in 8 non-human optimized codons) and a Flag tag fused to the C-terminus of EGFP with the linker EEMKA.

Claims Text - CLTX (37):

36. A method of screening for bioactive agents, said method comprising: a) combining a candidate bioactive agent and a cell comprising a fusion nucleic acid comprising i) a promoter ii) a codon optimized Renilla muelleri GFP; b) screening said cells for an altered phenotype.

Claims Text - CLTX (38):

37. A method of screening for bioactive agents, said method comprising a) combining a candidate bioactive agent and a cell comprising a fusion nucleic acid comprising i) a promoter ii) a codon optimized Ptilosarcus GFP; b) screening said cells for an altered phenotype.

Claims Text - CLTX (39):

38. A method of screening for bioactive agents according to claim 36, said fusion nucleic acid comprising: a) said promoter; b) said codon optimized Renilla muelleri GFP; c) a separation sequence; and d) a gene of interest.

Claims Text - CLTX (40):

39. A method of screening for bioactive agents according to claim 37, said fusion nucleic acid comprising: a) said promoter; b) said codon optimized Ptilosarcus GFP; c) a separation sequence; and d) a gene of interest.

Claims Text - CLTX (44):

43. A method according to claim 36 or 38 wherein said promoter comprises an IL-4 inducible .epsilon. promoter and said method further comprising: a) inducing said promoter with IL-4; and b) detecting said altered phenotype comprising absence or presence of expression of said codon optimized Renilla muelleri GFP.

Claims Text - CLTX (45):

44. A method according to claim 37 or 39 wherein said promoter comprises an IL-4 inducible .epsilon. promoter and said method further comprising: a) inducing said promoter with IL-4; and b) detecting said altered phenotype comprising absence or presence of expression of said codon optimized Ptilosarcus GFP.

PGPUB-DOCUMENT-NUMBER: 20030148262

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030148262 A1

TITLE: Chimeric alphavirus replicon particles

PUBLICATION-DATE: August 7, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 123101

DATE FILED: April 11, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295451 20010531 US

US-CL-CURRENT: 435/5, 424/192.1, 424/199.1, 424/204.1, 435/69.1  
, 435/69.7, 435/91.1, 530/300

ABSTRACT:

Chimeric alphaviruses and alphavirus replicon particles are provided including methods of making and using same. Specifically, alphavirus particles are provided having nucleic acid molecules derived from one or more alphaviruses and structural proteins (capsid and/or envelope) from at least two or more alphaviruses. Methods of making, using, and therapeutic preparations containing the chimeric alphavirus particle, are disclosed.

[0001] This application claims the benefit of U.S. Serial No. 60/295,451 filed May 31, 2001, which application is hereby incorporated by reference in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX (6):

[0042] A "nucleic acid" molecule can include, but is not limited to, prokaryotic sequences, eukaryotic mRNA or other RNA, cDNA from eukaryotic mRNA or other RNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA and includes modifications such as deletions, additions and substitutions (generally conservative in nature), to



the native sequence. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental. Modifications of polynucleotides may have any number of effects including, for example, facilitating expression of the polypeptide product in a host cell.

Detail Description Paragraph - DETX (219):

[0238] Chimera 1A and 1B were prepared by cleaving pVCR-DH, an intermediate construct from the re-assembly phase of the pVCR construction described (above), with MscI and Ascl. Into this vector was inserted either of two tripartite synthetic oligonucleotides coding, as described above, the last 80 bp or so of nsP4 with non-native codon usage, followed by the SIN packaging signal (in frame) and nsP4 termination codon, followed by the duplicated terminal 80 or 98 bp of native nsP4 sequence. The oligonucleotides were designed to provide synthetic full duplex strands that were treated in the same manner as was described earlier for the replicon synthesis. Sequence verified clones from this ligation were digested with MscI and Ascl, and the oligo fragment bearing the SIN packaging signal was substituted into the vector fragment of pVCR, digested similarly. The resulting final constructs for each was called pVCR/CHIMERA-1A and pVCR/CHIMERA-1B. To evaluate the functionality of these constructs, the GFP gene was cloned into each using the unique BbvCI and NotI sites downstream of the subgenomic promoter and the constructs were designated VCR-Chim1A-GFP and VCR-Chim1B-GFP respectively.

PGPUB-DOCUMENT-NUMBER: 20030108863

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030108863 A1

TITLE: Nodavirus-like DNA expression vector and uses therefor

PUBLICATION-DATE: June 12, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 230730

DATE FILED: November 4, 2002

RELATED-US-APPL-DATA:

child 10230730 A1 20021104

parent division-of 09595346 20000614 US GRANTED

parent-patent 6514757 US

US-CL-CURRENT: 435/5, 435/235.1 , 435/320.1 , 435/325 , 435/456 , 435/69.3  
, 536/23.72

ABSTRACT:

The present invention describes the production of a nodavirus-based DNA vector that drives abundant expression of foreign genes in a wide variety of cell types. The DNA plasmid is initially transcribed by a host-cell RNA polymerase to produce primary transcripts from which a nodaviral RNA-dependent RNA polymerase (RNA replicase) is translated. These primary transcripts are then amplified by the RNA replicase in an autonomous, cytoplasmic RNA replication. Such a vector is a useful addition to the current arsenal of expression vectors, and well suited to laboratory-scale and larger-scale expression of transcripts and/or proteins in eukaryotic cells.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This non-provisional patent application claims benefit of provisional patent application U.S. Serial No. 60/139,120, filed Jun. 14, 1999, now abandoned.

Detail Description Paragraph - DETX (23):

[0049] A "heterologous" region or gene of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. In another example, the coding sequence itself may not be found in nature (e.g., a cDNA, in which the genomic coding sequence contains introns, or synthetic sequences having codons different from the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

Detail Description Paragraph - DETX (61):

[0078] RNA-dependent RNA polymerase replication of the resultant transcript from the expression vector The next step is to harness RNA replication to drive the amplification of foreign mRNAs. To this end, the tandem delivery plasmid (FIG. 5) is modified by inserting a heterologous gene, in this case for experimental purposes, a reporter gene, as the 5'-proximal open reading frame in the RNA2 sequence (FIG. 6). Genes encoding firefly luciferase, lacZ, G418 resistance or the green fluorescent protein (GFP) are introduced into the downstream RNA2 replicon such that their open reading frames are flanked by the RNA2 cis-acting signals and positioned to initiate translation at the first AUG codon of the chimeric RNA2. To provide a versatile eukaryotic vector for general use, the insertion site for the reporter gene is flanked by several unique restriction sites for convenient cloning of other target genes, i.e., a multiple cloning site (MCS). The above-mentioned reporter genes were chosen because they can be readily assayed over a wide range of expression levels. For experimental controls, conventional monocistronic plasmids are also constructed that lack the RNA replicase gene but contain the same reporter genes flanked by the RNA2 cis-acting signals, placed directly downstream of an RNA pol II promoter. The levels and duration of reporter gene expression from the RNA replicon plasmids are compared with those from the control plasmids that rely solely on DNA-templated transcription for mRNA synthesis.

PGPUB-DOCUMENT-NUMBER: 20030051270

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030051270 A1

TITLE: Targeted chromosomal genomic alterations with modified  
single stranded oligonucleotides

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Gamper, Howard B.	Philadelphia	PA	US	
Rice, Michael C.	Newton	PA	US	

APPL-NO: 09/ 818875

DATE FILED: March 27, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60192176 20000327 US

non-provisional-of-provisional 60192179 20000327 US

non-provisional-of-provisional 60208538 20000601 US

non-provisional-of-provisional 60244989 20001030 US

US-CL-CURRENT: 800/21, 514/44 , 536/23.1

ABSTRACT:

Presented are methods and compositions for targeted chromosomal genomic alterations using modified single-stranded oligonucleotides. The oligonucleotides of the invention have at least one modified nuclease-resistant terminal region comprising phosphorothioate linkages, LNA analogs or 2'-O-Me base analogs.

----- KWIC -----

Brief Description of Drawings Paragraph - DRTX (8):

[0046] FIG. 7. Hygromycin-eGFP target plasmids. (A) Plasmid pAURHYG(ins)**GFP** contains a single base insertion mutation between nucleotides 136 and 137, at codon 46, of the Hygromycin B coding sequence (cds) which is transcribed from the constitutive ADHL promoter. The target sequence presented below indicates the deletion of an A and the substitution of a C for a T

directed by the oligonucleotides to re-establish the resistant phenotype. (B) Plasmid pAURHYG(rep)**GFP** contains a base substitution mutation introducing a G at nucleotide 137, at codon 46, of the Hygromycin B coding sequence (cds). The target sequence presented below the diagram indicates the amino acid conservative replacement of G with C, restoring gene function.

Detail Description Paragraph - DETX (4):

[0051] In this example, single-stranded and double-hairpin oligonucleotides with chimeric backbones (see FIG. 1 for structures (A and B) and sequences (C and D) of assay oligonucleotides) are used to correct a point mutation in the kanamycin gene of pK.sup.sm4021 (FIG. 2) or the tetracycline gene of pT.sup.s.DELTA.208 (FIG. 3). All kan oligonucleotides share the same 25 base sequence surrounding the target base identified for change, just as all tet oligonucleotides do. The sequence is given in FIGS. 1C and 1D. Each plasmid contains a functional ampicillin gene. Kanamycin gene function is restored when a G at position 4021 is converted to a C (via a substitution mutation); tetracycline gene function is restored when a deletion at position 208 is replaced by a C (via frameshift mutation). A separate plasmid, pAURNeo(-)FIAsH (FIG. 9), bearing the kan.sup.s gene is used in the cell culture experiments. This plasmid was constructed by inserting a synthetic expression cassette containing a neomycin phosphotransferase (kanamycin resistance) gene and an extended reading frame that encodes a receptor for the FIAsH ligand into the pAUR123 shuttle vector (Panvera Corp., Madison, Wis.). The resulting construct replicates in *S. cerevisiae* at low copy number, confers resistance to aureobasidinA and constitutively expresses either the Neo+/FIAsH fusion product (after alteration) or the truncated Neo-/FIAsH product (before alteration) from the ADH1 promoter. By extending the reading frame of this gene to code for a unique peptide sequence capable of binding a small ligand to form a fluorescent complex, restoration of expression by correction of the stop codon can be detected in real time using confocal microscopy. Additional constructs can be made to test additional gene alteration events.

PGPUB-DOCUMENT-NUMBER: 20030049841

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030049841 A1

TITLE: High throughput or capillary-based screening for a  
bioactivity or biomolecule

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Lafferty, William Michael	Encinitas	CA	US	

APPL-NO: 09/ 975036

DATE FILED: October 10, 2001

RELATED-US-APPL-DATA:

child 09975036 A1 20011010

parent continuation-in-part-of 09894956 20010627 US PENDING

child 09894956 20010627 US

parent continuation-in-part-of 09790321 20010221 US PENDING

child 09790321 20010221 US

parent continuation-in-part-of 09687219 20001012 US PENDING

child 09687219 20001012 US

parent continuation-in-part-of 09685432 20001010 US PENDING

child 09685432 20001010 US

parent continuation-in-part-of 09444112 19991122 US PENDING

child 09444112 19991122 US

parent continuation-in-part-of 09098206 19980616 US GRANTED

parent-patent 6174673 US

child 09098206 19980616 US

parent continuation-in-part-of 08876276 19970616 US PENDING

non-provisional-of-provisional 60309101 20010731 US

US-CL-CURRENT: 435/449

#### ABSTRACT:

Provided is a method of screening or enriching a sample containing polynucleotides from a mixed population of organisms. The method includes creating a DNA library from a plurality of nucleic acid sequences of a mixed population of organisms and separating clones containing a polynucleotide sequence of interest on an analyzer detects a detectable molecule on a probe or bioactive substrate. The analyzer includes FACS devices, SQUID devices and MCS devices. The separated or enrich library can then be further process by activity based screening or sequence based screening. In addition, the enriched sequence can be compared to a database and to identify sequences in the database which have homology to a clone in the library thereby obtaining a nucleic acid profile of the mixed population of organisms.

#### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. patent application Ser. No. 09/894,956, filed Jun. 27, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/790,321, filed Feb. 21, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/687,219, filed Oct. 12, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/685,432, filed Oct. 10, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/444,112, filed Nov. 22, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/098,206, filed Jun. 16, 1998, now U.S. Pat. No. 6,174,673, which is a continuation-in-part of U.S. patent application Ser. No. 08/876,276, filed Jun. 16, 1997; this application also claims priority to U.S. patent application Ser. No. 09/738,871, filed Dec. 14, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/685,432, filed Oct. 10, 2000, which is a continuation in part of U.S. patent application Ser. No. 09/444,112, filed Nov. 22, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/098,206, filed Jun. 16, 1998, now U.S. Pat. No. 6,174,673, which is a continuation-in-part of U.S. patent application Ser. No. 08/876,276, filed Jun. 16, 1997; this application also claims priority to U.S. Provisional Application 60/309,101, the contents of which are all incorporated by reference in their entirety herein.

----- KWIC -----

#### Detail Description Paragraph - DETX (12):

[0111] In one aspect, this high throughput culturing method followed by sorting (e.g., FACS) screening (e.g., biopanning), allows for identification of gene targets. It may be desirable to screen for nucleic acids encoding virtually any protein or any bioactivity and to compare such nucleic acids among various species of organisms in a sample (e.g., study polyketide

sequences from a mixed population). In another aspect, nucleic acid derived from high throughput culturing of organisms can be obtained for further study or for generation of a library. Such nucleic acid can be pooled and a library created, or alternatively, individual libraries from clonal populations of organisms can be generated and then nucleic acid pooled from those libraries to generate a more complex library. The libraries generated as described herein can be utilized for the discovery of biomolecules (e.g., nucleic acid or bioactivities) or for evolving nucleic acid molecules identified by the high throughput culturing methods described in the present invention. Such evolution methods are known in the art or described herein, such as, shuffling, cassette mutagenesis, recursive ensemble mutagenesis, sexual PCR, directed evolution, exonuclease-mediated reassembly, codon site-saturation mutagenesis, amino acid site-saturation mutagenesis, gene site saturation mutagenesis, introduction of mutations by non-stochastic polynucleotide reassembly methods, synthetic ligation polynucleotide reassembly, gene reassembly, oligonucleotide-directed saturation mutagenesis, in vivo reassortment of polynucleotide sequences having partial homology, naturally occurring recombination processes which reduce sequence complexity, and any combination thereof.

Detail Description Paragraph - DETX (104):

[0203] Alternatively, it may be desirable to variegate a polynucleotide sequence obtained, identified or cloned as described herein. Such variegation can modify the polynucleotide sequence in order to modify (e.g., increase or decrease) the encoded polypeptide's activity, specificity, affinity, function, etc. Such evolution methods are known in the art or described herein, such as, shuffling, cassette mutagenesis, recursive ensemble mutagenesis, sexual PCR, directed evolution, exonuclease-mediated reassembly, codon site-saturation mutagenesis, amino acid site-saturation mutagenesis, gene site saturation mutagenesis, introduction of mutations by non-stochastic polynucleotide reassembly methods, synthetic ligation polynucleotide reassembly, gene reassembly, oligonucleotide-directed saturation mutagenesis, in vivo reassortment of polynucleotide sequences having partial homology, naturally occurring recombination processes which reduce sequence complexity, and any combination thereof.

Detail Description Paragraph - DETX (522):

[0596] There are several methods that can be used to optimize for increased pathway expression within the microdrops. For easy detection of maximal expression, we will construct a transposon containing a promoter-less GFP. The enhanced GFP optimized for eukaryotes will be used as it has a codon bias for high GC organisms. Transposition into a known pathway (e.g., actinorhodin) will be done in vitro and the vector containing the pathway purified. The transposants will be introduced into an E. coli host, screened for clones that express GFP, and positive clones isolated on the flow cytometer. With the transfer of the promoter-less gene for GFP into the pathway, increased fluorescence within the cells would suggest transcription of the pathway using the endogenous promoters located within the pathway. This clone will be used as a tool for quick detection of upregulation in pathway expression due to changes in the experimental conditions.



PGPUB-DOCUMENT-NUMBER: 20030049229

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030049229 A1

TITLE: Molecular clones with mutated HIV gag/pol, SIV gag and  
SIV env genes

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Pavlakis, George N.	Rockville	MD	US	

APPL-NO: 10/ 263020

DATE FILED: October 2, 2002

RELATED-US-APPL-DATA:

child 10263020 A1 20021002

parent continuation-of 09872733 20010601 US PENDING

child 09872733 20010601 US

parent continuation-in-part-of PCT/US00/34985 20001222 US PENDING

non-provisional-of-provisional 60173036 19991223 US

US-CL-CURRENT: 424/93.2, 435/235.1 , 435/252.33 , 435/366 , 435/456  
, 536/23.1

ABSTRACT:

Nucleic acid constructs containing HIV-1 gag/pol and SIV gag or SIV env genes which have been mutated to remove or reduce inhibitory/instability sequences are disclosed. Viral particles and host cells containing these constructs and/or viral particles are also disclosed. The exemplified constructs and viral particles of the invention may be useful in gene therapy for numerous disorders, including HIV infection, or as a vaccine for HIV-1 immunotherapy and immunoprophylaxis.

----- KWIC -----

Detail Description Paragraph - DETX (7):

[0058] The HIV gag/pol clone and SIV gag clone of the invention were made using the method for eliminating inhibitory/instability regions from a gene as

described in U.S. Pat. No. 6,174,666, and also in related U.S. Pat. Nos. 5,972,596 and 5,965,726, which are incorporated by reference herein. This method does not require the identification of the exact location or knowledge of the mechanism of function of the INS. Generally, the mutations are such that the amino acid sequence encoded by the mRNA is unchanged, although conservative and non-conservative amino acid substitutions are also envisioned where the protein encoded by the mutated gene is substantially similar to the protein encoded by the non-mutated gene. The mutated genes can be synthetic (e.g., synthesized by chemical synthesis), semi-synthetic (e.g., a combination of genomic DNA, cDNA, or PCR amplified DNA and synthetic DNA), or recombinantly produced. The genes also may optionally not contain introns. The nucleic acids of the invention may also contain Rev-independent fragments of these genes which retain the desired function (e.g., for antigenicity of Gag or Pol, particle formation (Gag) or enzymatic activity (Pol)), or they may also contain Rev-independent variants which have been mutated so that the encoded protein loses a function that is unwanted in certain circumstances. In the latter case, for example, the gene may be modified to encode mutations (at the amino acid level) in the active site of reverse transcriptase or integrase proteins to prevent reverse transcription or integration. Rev-independent fragments of the gag gene are described in U.S. patent application Ser. No. 07/858,747, filed Mar. 27, 1992, and also in related U.S. Pat. Nos. 5,972,596 and 5,965,726, which are incorporated by reference herein.

Detail Description Paragraph - DETX (68):

[0109] In the packaging construct shown in FIG. 5, "CMV" denotes the cytomegalovirus promoter. "Gag" denotes the gag gene, which generates components of the virion core, "Pro" denotes "protease" "RT" denotes "reverse transcriptase," "Int" denotes "integrase" and "BGH poly (A)" denotes the bovine growth hormone polyadenylation signal. The protease, reverse transcriptase, and integrase genes comprise the "pol" gene. In transfer construct 1, "LTR" denotes the HIV "long terminal repeat", which contains a HIV promoter; "mSD" denotes "mutated splice donor site," which is present in the construct so that splicing of the RNA transcript does not occur; ".psi." denotes the encapsidation signal; "wGA" denotes part of the wild-type gag gene which contains sequences believed to be necessary for encapsidation; "X" indicates that the ATG codon of the partial gag gene sequence is mutated so that translation of this gene does not occur; "CMV" denotes the cytomegalovirus promoter and luciferase is used as a reporter gene. Luciferase can be replaced with any gene of interest. Another HIV LTR is present at the 3' end of transfer construct 1. Replacement of this LTR in constructs such as the transfer construct 1, 2, or 3 with a promoter-enhancer deleted HIV LTR leads to inactivation of LTR after integration. Transfer construct 2 is similar to transfer construct 1, the difference being that a mutated part of the gag gene (denoted "mGa") is used instead of the wild-type part of the gag gene. Transfer construct 3 (pm2BCwCNluci) has different mutations at the 5' splice site and has an intact ATG codon so that translation of part of the mutated gag gene occurs. Transfer construct 3 also has a 5' CMV promoter instead of a 5' LTR promoter. This construct is expressed independent of the presence of HIV Tat protein. The transfer constructs expressed from the LTR promoter are partially dependent on Tat protein. In 293 cells significant expression can be achieved in the absence of Tat. See, e.g., Valentin et al., Proc. Natl Acad.

Sci. U.S.A. 95:8886-91 (1988).

PGPUB-DOCUMENT-NUMBER: 20020168707

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020168707 A1

TITLE: SYNTHETIC GENES AND GENETIC CONSTRUCTS COMPRISING SAME

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
GRAHAM, MICHAEL	WAYNE	ST. LUCIA		AU

APPL-NO: 09/ 100812

DATE FILED: June 19, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
AU	PP2292	1998AU-PP2292	March 20, 1998

US-CL-CURRENT: 435/69.1, 424/93.2 , 435/320.1 , 435/325 , 435/455 , 514/44 , 536/23.1 , 536/23.5

ABSTRACT:

The present invention relates generally to synthetic genes for modifying endogenous gene expression in a cell, tissue or organ of a transgenic organism, in particular a transgenic animal or plant. More particularly, the present invention provides novel synthetic genes and genetic constructs which are capable of repressing delaying or otherwise reducing the expression of an endogenous gene or a target gene in an organism when introduced thereto.

----- KWIC -----

Detail Description Paragraph - DETX (16):

[0057] Generally, a gene of the invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions without affecting its ability to modify target gene expression. Nucleotide insertional derivatives of the synthetic gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable

screening of the resulting product.

Detail Description Paragraph - DETX (26):

[0067] Alternatively, the structural gene may comprise a nucleotide sequence which does not encode an amino acid sequence or more commonly, comprises one or more open reading frames which encode one or more peptides, oligopeptides or polypeptides which are unrelated at the amino acid sequence level to the amino acid sequence encoded by the target gene. For example, the mRNA product of the structural gene may be inserted into the synthetic gene of the invention so as to alter or disrupt the reading frame of the structural gene and produce one or more frame shift mutations in the translation product thereof relative to the translation product encoded by the target gene, notwithstanding a substantial identity between the structural gene and the target gene on the one hand and the corresponding mRNA products of the structural gene and the target gene on the other hand. Such effects may be produced by introducing one or two nucleotide substitutions or deletions into the structural gene, relative to the target gene sequence or alternatively, by introducing a translation start codon 5'-ATG-3' upstream of any nucleotide in the structural gene which occurs at a particular position in a codon of the corresponding target gene such that the position of said nucleotide in the codon of the structural gene is altered.

Detail Description Paragraph - DETX (183):

[0208] Plasmid pCMVLacI.TYR.OPRSV1.GFP (FIG. 28) is a dual construct in which the CMV IE promoter drives expression of the lacI gene and the mRNA of the mouse tyrosinase cDNA or a fragment thereof, whilst the OPRSV1 promoter drives expression of GFP operably under control of the lacI gene. The construct is designed such that the mouse tyrosinase gene is fused to the 3' untranslated region of the lacI gene via a unique BsaB1 cloning site. This cloning site is located after the stop codon of the lacI coding sequence, but before the SV40 polyadenylation signal. The construct also contains the hygromycin-resistance gene as a selection marker.

PGPUB-DOCUMENT-NUMBER: 20020142297

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020142297 A1

TITLE: Compositions and methods for imaging gene expression

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bogdanov, Alexei A.	Arlington	MA	US	
Weissleder, Ralph	Charlestown	MA	US	
Simonova, Maria	Boston	MA	US	

APPL-NO: 09/ 739068

DATE FILED: December 18, 2000

RELATED-US-APPL-DATA:

child 09739068 A1 20001218

parent continuation-of 09015366 19980129 US ABANDONED

non-provisional-of-provisional 60037350 19970131 US

US-CL-CURRENT: 435/6, 435/7.1 , 530/400

ABSTRACT:

Described are short peptide sequences, termed recombinant peptide chelates (RPCs), and the imaging marker genes that encode them. The RPCs can be expressed in parallel with the expression of any other desired gene (e.g., a therapeutic gene), and used to easily confirm the expression of the therapeutic gene product. The RPCs are expressed in the cell or on the cell surface concurrently with the therapeutic gene product, and can be assayed by standard imaging techniques.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from provisional application 60/037,350, filed Jan. 31, 1997.

----- KWIC -----

Detail Description Paragraph - DETX (43):

[0066] To create a new BamHI restriction site for further linkage with a

tyrosinase fragment, the following **mutagenic** oligonucleotide was chosen: 5'-GCCTGCGACCTGGGGATCCCCGCCGGCA CC-3' (SEQ ID NO: 9), where the induced **mutations** are shown in bold-face type. Another **synthetic oligonucleotide from the PLAP 3'-terminal end has the sequence**: 5'-CTCAGGGAGCAGTGGCGTCTCCAGCAGCAG-3' (SEQ ID NO: 10). This small PCR product therefore included a DNA sequence corresponding to a 35 residue peptide encompassing the 29 amino acid PLAP hydrophobic region, the Asp-484 GPI addition site, the five amino acids upstream of Asp-484, and a stop codon site.

Detail Description Paragraph - DETX (89):

[0102] A spontaneous deletion of the 5'-end corresponding to eight N-terminal amino acids was revealed in one of the isolates (clone 6). In this clone, the AUG **codon of GFP**-P fusion protein is immediately adjacent to the BSKS(+) T3 **promoter** region. The corresponding translation of the fusion protein sequence is represented by: TMITPSAQL TLTKGMet(**GFP**)LGGGGCGGGCGI (SEQ ID NO: 21-**GFP**)-SEQ ID NO: 17). These two variants of "hydrophobic" fusion proteins were studied separately for their ability to form complexes with oxotetrate.

PGPUB-DOCUMENT-NUMBER: 20020107362

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020107362 A1

TITLE: Novel fluorescent proteins

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Thastrup, Ole	Birkerød		DK	
Tullin, Søren	Soborg		DK	
Poulsen, Lars Kongsbak	Holte		DK	
Bjorn, Sara Petersen	Lyngby		DK	

APPL-NO: 09/ 872364

DATE FILED: June 1, 2001

RELATED-US-APPL-DATA:

child 09872364 A1 20010601

parent continuation-of 09619310 20000719 US PENDING

child 09619310 20000719 US

parent continuation-of 08819612 19970317 US GRANTED

parent-patent 6172188 US

child 08819612 19970317 US

parent continuation-of PCT/DK96/00051 19960131 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1065/95	1995DK-1065/95	September 22, 1995

US-CL-CURRENT: 530/350, 435/320.1, 435/325, 435/69.1, 536/23.2

ABSTRACT:

The present invention relates to novel variants of the fluorescent protein GFP having improved fluorescence properties.

----- KWIC -----



Detail Description Paragraph - DETX (3):

[0070] Briefly, total RNA, isolated from *A. victoria* by a standard procedure (Sambrook et al., Molecular Cloning. 2., eds. (1989) (Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.), 7.19-7.22) was converted into cDNA by using the AMV reverse transcriptase (Promega, Madison, Wis., USA) as recommended by the manufacturer. The cDNA was then PCR amplified, using PCR primers designed on the basis of a previously published GFP sequence (Prasher et al., Gene 111 (1992), 229-223; GenBank accession No. M62653) together with the UITma.TM. polymerase (Perkin Elmer, Foster City, Calif., USA). The sequences of the primers were: GFP2: TGG AATAAGCTTTATGAGTAAAGGAGAAGAACTTTT and GFP-1: AAGAATTCGGATCCCTTTAGTGCAATTGGAAGTCT Restriction endonuclease sites inserted in the 5' (a HindIII site) and 3' (EcoRI and BamHI sites) primers facilitated the cloning of the PCR amplified GFP cDNA into a slightly modified pUC19 vector. The details of the construction are as follows: LacZ Shine-Dalgarno AGGA, immediately followed by the 5' HindIII site plus an extra T and the GFP ATG codon, giving the following DNA sequence at the lacZ-promoter GFP fusion point: P.sub.LacZ-AGGAAAGCTTTATG-GFP. At the 3' end of the GFP cDNA, the 5 base pair corresponding to nucleotide 770 in the published GFP sequence (GenBank accession No. M62653) was fused to the EcoRI site of the pUC19 multiple cloning site (MCS) through a PCR generated BamHI, EcoRI linker region).

Detail Description Paragraph - DETX (14):

[0080] The S65T-GFP mutation was described by Heim et al (Nature vol.373 pp. 663-664, 1995). F64L-S65T-GFP was constructed as follows: An E.coli expression vector containing Y66H-GFP was digested with restriction enzymes Nco1 and Xba1. The recognition sequence of Nco1 is located at position 173 and the recognition sequence of Xba1 is located at position 221 in the F64L-Y66H-GFP sequence listed below. The large Nco1-Xba1 vector fragment was isolated and ligated with a synthetic Nco1-Xba1 DNA linker of the following sequence:

PGPUB-DOCUMENT-NUMBER: 20020090605

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020090605 A1

TITLE: Methods for identifying, characterizing, and evolving  
cell-type specific cis regulatory elements

PUBLICATION-DATE: July 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kamb, Carl Alexander	Salt Lake City	UT	US	

APPL-NO: 09/ 935929

DATE FILED: August 23, 2001

RELATED-US-APPL-DATA:

child 09935929 A1 20010823

parent continuation-of 09378420 19990820 US ABANDONED

child 09378420 19990820 US

parent continuation-in-part-of 08800664 19970214 US PENDING

US-CL-CURRENT: 435/4, 435/6 , 435/7.23

ABSTRACT:

The invention provides methods for efficient and rapid identification of cis-acting nucleic acid sequences that act in a cell-type or cell-state specific manner to stimulate or repress the expression of linked genes or other neighboring sequences. The invention also provides methods for evolving novel regulatory sequences by in vitro manipulation of naturally occurring or synthetic cis acting nucleic acid sequences followed by screening and counterscreening steps. Furthermore, the invention provides methods for determining the mechanism by which cell-type specific cis regulatory sequences confer cell-specific expression. Also provided are diagnostic methods based on the use of cell-specific cis regulatory sequences.

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of priority application U.S. Ser. Nos. 08/800,664, "Methods for identifying, characterizing and evolving cell type-specific cis regulatory elements, the disclosure of which is expressly incorporated by reference herein in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX (46):

[0081] The invention permits identification of novel regulatory elements that involve sequence variants, combinations and permutations of natural promoters, enhancers, negative regulatory sequence elements, and/or synthetic DNA sequences. The methods used to create such non-natural sequences include the following types of manipulations. Sub-library sequences that have a particular activity are either mutated in vitro by any of several methods known in the art, or rejoined with other natural or non-natural fragments by ligation, or digestion and re-ligation (Ausubel F. M., Brent R., et al., 1996). These new sub-libraries are passaged through the same host cells (or different cell types) and the selection and counter selection steps are repeated. The method thus permits the evolution of more desirable properties in a series of steps that involve manipulation of library sequences in vitro followed by selection in vivo. Thus, it is possible to evolve, e.g., a cis sequence that is more completely "off" in one cell type and more active in another.

Detail Description Paragraph - DETX (67):

[0098] Construction of a promoterless mammalian expression vector pEGFP-C1 (Clontech Laboratories, Palo Alto, Calif.; GenBank accession number U55763) is used as a starting material to construct the parental vector. It contains the GFP coding sequence flanked by a CMV promoter/enhancer on its 5' side, and the SV40 T-Antigen gene polyadenylation signal on the 3' side (FIG. 1). This vector is modified so that upstream of the GFP translational start codon are sequences that either include part of the functional promoter (the TATA box from the CMV promoter, generated by trimming pEGP-C1 to a position -63 base pairs from the translational start codon), or sequences completely missing the promoter (trimmed to -10 base pairs upstream of the GFP start). These two crippled ("dead") expression vectors lack sequences necessary for GFP expression in most mammalian cells. The vector is further engineered so that restriction enzyme recognition sites, useful for inserting library fragments, are introduced at positions -63 and -69.

PGPUB-DOCUMENT-NUMBER: 20020016980

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020016980 A1

TITLE: Transgenic plants incorporating traits of *Zostera marina*

PUBLICATION-DATE: February 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Alberte, Randall S.	Falmouth	ME	US	
Smith, Robert	Falmouth	ME	US	

APPL-NO: 09/ 854122

DATE FILED: May 10, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60202529 20000510 US

US-CL-CURRENT: 800/289, 536/23.6 , 800/278

ABSTRACT:

The invention provides methods and compositions related to transgenic plants which incorporate genetic traits of the marine eelgrass *Zostera marina*. These traits include pathogen resistance, which may be conferred by stimulating zosteric acid biosynthesis, and root anoxia resistance, which may be conferred by introducing one or more anoxia-induced or anoxia-resistance genes.

----- KWIC -----

Detail Description Paragraph - DETX (39):

[0075] The term "marker" or "marker sequence" or similar phrase means any gene that produces a selectable genotype or preferably a selectable phenotype. It includes such examples as the neo gene, green fluorescent protein (**GFP**) gene, TK gene, b-galactosidase gene, etc. The marker sequence may be any sequence known to those skilled in the art that serves these purposes, although typically the marker sequence will be a sequence encoding a protein that confers a selectable trait, such as an antibiotic resistance gene, or an enzyme that can be detected and that is not typically found in the cell. The marker sequence may also include regulatory regions such as a **promoter** or enhancer that regulates the expression of that protein. However, it is also possible to transcribe the marker using endogenous regulatory sequences. In one embodiment of the present invention, the marker facilitates separation of transfected from

untransfected cells by fluorescence activated cell sorting, for example by the use of a fluorescently labeled antibody or the expression of a fluorescent protein such as GFP. Other DNA sequences that facilitate expression of marker genes may also be incorporated into the DNA constructs of the present invention. These sequences include, but are not limited to transcription initiation and termination signals, translation signals, post-translational modification signals, intron splicing junctions, ribosome binding sites, and polyadenylation signals, to name a few. The marker sequence may also be used to append sequence to the target gene. For example, it may be used to add a stop codon to truncate IL-1RN translation. The use of selectable markers is well known in the art and need not be detailed herein. The term "modulation" as used herein refers to both upregulation (i.e., activation or stimulation (e.g., by agonizing or potentiating)) and downregulation (i.e. inhibition or suppression (e.g., by antagonizing, decreasing or inhibiting)).

Detail Description Paragraph - DETX (80):

[0116] The invention still further provides for mutated derivatives of native sulfotransferase gene sequences from an organism such as *Zostera marina*. For example, the gene sequence of a *Zostera marina* sulfotransferase may be altered so as to optimize codon utilization and increase expression in the target host plant. In addition, a synthetic sulfotransferase encoding gene may be used in the method of the invention. For example, catalytic antibodies, designed to bind to a phenol sulfotransferase catalytic intermediate antigen, may be generated using methods known in the art (see e.g. Jacobsen and Schultz (1995) *Curr Opin Struct Biol* 5: 818). The nucleic acid encoding these antibodies with phenol sulfotransferase catalytic activity may then be introduced into the target host plant to provide a "synthetic" PST activity of the invention.

PGPUB-DOCUMENT-NUMBER: 20020015979

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020015979 A1

TITLE: VEHICLES FOR STABLE TRANSFER OF GREEN FLUORESCENT  
PROTEIN GENE AND METHODS OF USE FOR SAME

PUBLICATION-DATE: February 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
LINK, CHARLES J. JR.	CLIVE	IA	US	
LEVY, JOHN P.	WEST DES MOINES	IA	US	
WANG, SUMING	DES MOINES	IA	US	
SEREGINA, TATIANA	WEST DES MOINES	IA	US	

APPL-NO: 08/ 786531

DATE FILED: January 21, 1997

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60010371 19960122 US

US-CL-CURRENT: 435/69.1, 435/189, 435/252.3, 435/320.1, 435/69.7  
, 435/70.3, 435/70.4, 536/23.4, 536/23.5, 536/24.1  
, 536/24.2

ABSTRACT:

The present invention describes an efficient retroviral or viral based method that allows easy and quick identification of gene transfer in living, transduced mammalian cells. Retroviral and viral vector producer cells were generated containing a gene for an improved humanized red-shifted, Green Fluorescent Protein (hRGFP) which increases the resulting fluorescence yield after excitation. This humanized, red-shifted GFP (hRGFP) gene was cloned into several vectors and transfected into various packaging cell lines to produce vibrant green fluorescence after excitation with blue light at 450-490 nm. These vectors represent a substantial advance over currently available gene transfer marking systems or wild-type GFP marker systems none of which have been stably transfected into cells.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part of provisional application Serial. No. 60/010,371 filed Jan. 22, 1996.

----- KWIC -----

Detail Description Paragraph - DETX (32):

[0078] The instant invention demonstrates the effectiveness of a humanized, red shifted mutant GFP by retroviral and viral mediated gene transfer into human tumor cells and murine fibroblasts. A few molecular genetics groups have now reported mutations of the wild-type GFP gene which can generate GFP gene products with modified excitation and emission spectra. See for e.g. Heim, R., et al. (1994) Proc Natl Acad Sci, USA 91:12501-12504. The longer wavelength excitation peak (475 nm) of native A. Victoria GFP has lower amplitude than its shorter wavelength excitation peak (470-490 nm) with fluorescence amplitudes from 4-6 fold greater than from the wild-type gene product. Heim, R. (1995) Nature 373:663-664. Interestingly, this mutant also had more rapid formation of the fluorochrome. Id. Furthermore, the mutate, red shifted GFP had its codons modified to usage common in mammals (Dr. Sergei Zolotukhin and Dr. Nicholas Muzyczka, University of Florida, unpublished results). The inventors have evaluated this humanized version of a serine-65 to threonine codon mutant that demonstrates excitation at 490 nm and emissions at 510 nm in current gene transfer experiments. Comparisons between the wild-type GFP and the humanized, serine-65 red shifted mutant (hRGFP) demonstrated substantial improvement in fluorescence expression after either transfection and retroviral mediated GFP gene transfer (Table 1).

Detail Description Paragraph - DETX (35):

[0081] The present invention demonstrates the ability to analyze and easily detect living, retroviral transduced cells without the need for cell fixation or antibodies. This allows for the clinical application of this marker in living tissue by using the appropriate enhancer/promoter or targeted transduction procedure. This may be particularly useful in bone marrow processing, lymphocyte sorting, and other applications requiring the FACS analysis of living cells. Further, since the GFP gene has not been shown to have any cytotoxicity in vitro, it is expected that the GFP gene will be safe for in vivo use. Future directions will lead to a wider range of useful GFP based mutant proteins with well defined fluorophores with characterized excitation and emission spectra, using mammalian codon usage. For example, Heim and colleagues subjected the native GFP sequence to random mutations in bacteria and developed a series of interesting mutants with altered excitation peaks and found that a substitution of Tyrosine-66 to histidine generated a mutant GFP that demonstrated blue fluorescent emission. Heim, R., et al. (1994) Proc Natl Acad Sci, USA 91:12501-12504. This may permit two color separation and analysis of living cells by FACS.

Detail Description Paragraph - DETX (56):

[0099] These results demonstrate the effectiveness of a humanized, red-shifted mutant GFP by retroviral mediated gene transfer into human tumor cells and murine fibroblasts.

Detail Description Table CWU - DETL (2):

2TABLE 2 SEQUENCE AND RESTRICTION SITE INFORMATION (SEQ ID NO. 1)  
phGFP-S65T Humanized GFP Vector GenBank Accession #: U43284 Location of features Human cytomegalovirus (CMV) immediate early promoter: 152-739 Enhancer region: 229-635 TATA box: 724-730 T7 promoter: 784-803 Green fluorescent protein gene (S65T variant) Start codon (ATG): 826-828; Stop codon: 1543-1545 GFP fluorescent chromophore: 1021-1029 SV40 small t antigen intron: 1642-1706 SV40 early mRNA polyadenylation signal Polyadenylation signals: 2312-2317 & 2341-2346 mRNA 3' ends: 2350 & 2362 SV40 origin of replication: 2805-2740 pBR322 plasmid replication origin: 2767-3347 M13 single-strand DNA origin: 3367-3934 Synthetic supF gene: 4145-3947 Geneology From To 1549 811 pCDM7 vector backbone (Not I-Hind III) 1 151 Fragment from the Rous Sarcoma Virus (RSV) LTR 152 738 Fragment from Human Cytomegalovirus (CMV) containing the immediate early promoter 812 1548 Synthetic GFP gene using optimal human codons 817 829 Synthetic Kozak consensus translation initiation sequence 829 831 Additional valine not present in wt GFP 1021 1023 865T mutation in GFP chromophore replacing serine 65 with threonine [Heim, R. et al. (1995) Nature 373:663-664] 1565 2174 Fragment from SV40 providing small antigen intron 2175 2415 Fragment from SV40 providing polyadenylation signals 2416 2759 Fragment from SV40 providing origin of replication 2767 3347 Fragment from pBR322 providing origin of replication 3367 3934 Fragment from M13 providing single-stranded DNA origin 3947 4145 Synthetic supF gene Propagation in E. coli Suitable host strain: MC1061/P3 Selectable Marker: The supF gene confers resistance to ampicillin (25-40 .mu.g/ml) and tetracycline (7.5-10 .mu.g/ml) to MC1061/P3 due to expression of a tRNA that suppresses amber mutations in the ampicillin and tetracycline genes on the P3 plasmid. E. coli replication origin: pBR322 (rop.sup.-) Copy number: = 100-200 Plasmid incompatibility group: pMB1/ColE1



PGPUB-DOCUMENT-NUMBER: 20010036655

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010036655 A1

TITLE: Molecular clones with mutated HIV gag/pol, SIV gag and  
SIV env genes

PUBLICATION-DATE: November 1, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Pavlakakis, George N.	Rockville	MD	US	

APPL-NO: 09/ 872733

DATE FILED: June 1, 2001

RELATED-US-APPL-DATA:

child 09872733 A1 20010601

parent continuation-in-part-of PCT/US00/34985 20001222 US UNKNOWN

non-provisional-of-provisional 60173036 19991223 US

US-CL-CURRENT: 435/235.1, 424/93.21 , 435/366

ABSTRACT:

Nucleic acid constructs containing HIV-1 gag/pol and SIV gag or SIV genes which have been mutated to remove or reduce inhibitory/instability sequences are disclosed. Viral particles and host cells containing these constructs and/or viral particles are also disclosed. The exemplified constructs and viral particles of the invention may be useful in gene therapy for numerous disorders, including HIV infection, or as a vaccine for HIV-1 immunotherapy and immunoprophylaxis.

----- KWIC -----

Detail Description Paragraph - DETX (7):

[0058] The HIV gag/pol clone and SIV gag clone of the invention were made using the method for eliminating inhibitory/instability regions from a gene as described in U.S. Pat. No. 6,174,666, and also in related U.S. Pat. Nos. 5,972,596 and 5,965,726, which are incorporated by reference herein. This method does not require the identification of the exact location or knowledge of the mechanism of function of the INS. Generally, the mutations are such that the amino acid sequence encoded by the mRNA is unchanged, although

conservative and non-conservative amino acid substitutions are also envisioned where the protein encoded by the mutated gene is substantially similar to the protein encoded by the non-mutated gene. The mutated genes can be synthetic (e.g., synthesized by chemical synthesis), semi-synthetic (e.g., a combination of genomic DNA, cDNA, or PCR amplified DNA and synthetic DNA), or recombinantly produced. The genes also may optionally not contain introns. The nucleic acids of the invention may also contain Rev-independent fragments of these genes which retain the desired function (e.g., for antigenicity of Gag or Pol, particle formation (Gag) or enzymatic activity (Pol)), or they may also contain Rev-independent variants which have been mutated so that the encoded protein loses a function that is unwanted in certain circumstances. In the latter case, for example, the gene may be modified to encode mutations (at the amino acid level) in the active site of reverse transcriptase or integrase proteins to prevent reverse transcription or integration. Rev-independent fragments of the gag gene are described in U.S. patent application Ser. No. 07/858,747, filed Mar. 27, 1992, and also in related U.S. Pat. Nos. 5,972,596 and 5,965,726, which are incorporated by reference herein.

Detail Description Paragraph - DETX (68):

[0109] In the packaging construct shown in FIG. 5, "CMV" denotes the cytomegalovirus promoter, "Gag" denotes the gag gene, which generates components of the virion core, "Pro" denotes "protease" "RT" denotes "reverse transcriptase," "Int" denotes "integrase" and "BGH poly (A)" denotes the bovine growth hormone polyadenylation signal. The protease, reverse transcriptase, and integrase genes comprise the "pol" gene. In transfer construct 1, "LTR" denotes the HIV "long terminal repeat", which contains a HIV promoter; "mSD" denotes "mutated splice donor site," which is present in the construct so that splicing of the RNA transcript does not occur; ".psi." denotes the encapsidation signal; "wGA" denotes part of the wild-type gag gene which contains sequences believed to be necessary for encapsidation; "X" indicates that the ATG codon of the partial gag gene sequence is mutated so that translation of this gene does not occur; "CMV" denotes the cytomegalovirus promoter and luciferase is used as a reporter gene. Luciferase can be replaced with any gene of interest. Another HIV LTR is present at the 3' end of transfer construct 1. Replacement of this LTR in constructs such as the transfer construct 1, 2, or 3 with a promoter-enhancer deleted HIV LTR leads to inactivation of LTR after integration. Transfer construct 2 is similar to transfer construct 1, the difference being that a mutated part of the gag gene (denoted "mGa") is used instead of the wild-type part of the gag gene. Transfer construct 3 (pm2BCwCNluci) has different mutations at the 5' splice site and has an intact ATG codon so that translation of part of the mutated gag gene occurs. Transfer construct 3 also has a 5' CMV promoter instead of a 5' LTR promoter. This construct is expressed independent of the presence of HIV Tat protein. The transfer constructs expressed from the LTR promoter are partially dependent on Tat protein. In 293 cells significant expression can be achieved in the absence of Tat. See, e.g., Valentin et al., Proc. Natl Acad. Sci. U S A. 95:8886-91 (1988).

US-PAT-NO: 6656706

DOCUMENT-IDENTIFIER: US 6656706 B2

TITLE: Molecular clones with mutated HIV gag/pol, SIV gag and  
SIV env genes

DATE-ISSUED: December 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pavlakis; George N.	Rockville	MD	N/A	N/A

APPL-NO: 09/ 872733

DATE FILED: June 1, 2001

PARENT-CASE:

This is continuation-in-part of international application No.  
PCT/US00/34985, filed Dec. 22, 2000 which claims benefit to Provisional  
application No. 60/173,036, filed Dec. 23, 1999.

US-CL-CURRENT: 435/69.1, 424/93.2, 435/252.3, 435/320.1, 435/325  
, 435/455, 435/91.4, 514/44, 536/23.1

ABSTRACT:

Nucleic acid constructs containing HIV-1 gag/pol and SIV gag or SIV env genes which have been mutated to remove or reduce inhibitory/instability sequences are disclosed. Viral particles and host cells containing these constructs and/or viral particles are also disclosed. The exemplified constructs and viral particles of the invention may be useful in gene therapy for numerous disorders, including HIV infection, or as a vaccine for HIV-1 immunotherapy and immunoprophylaxis.

22 Claims, 45 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 45

----- KWIC -----

Detailed Description Text - DETX (7):

The HIV gag/pol clone and SIV gag clone of the invention were made using the method for eliminating inhibitory/instability regions from a gene as described in U.S. Pat. No. 6,174,666, and also in related U.S. Pat. Nos. 5,972,596

and 5,965,726, which are incorporated by reference herein. This method does not require the identification of the exact location or knowledge of the mechanism of function of the INS. Generally, the mutations are such that the amino acid sequence encoded by the mRNA is unchanged, although conservative and non-conservative amino acid substitutions are also envisioned where the protein encoded by the mutated gene is substantially similar to the protein encoded by the non-mutated gene. The mutated genes can be synthetic (e.g., synthesized by chemical synthesis), semi-synthetic (e.g., a combination of genomic DNA, cDNA, or PCR amplified DNA and synthetic DNA), or recombinantly produced. The genes also may optionally not contain introns. The nucleic acids of the invention may also contain Rev-independent fragments of these genes which retain the desired function (e.g., for antigenicity of Gag or Pol, particle formation (Gag) or enzymatic activity (Pol)), or they may also contain Rev-independent variants which have been mutated so that the encoded protein loses a function that is unwanted in certain circumstances. In the latter case, for example, the gene may be modified to encode mutations (at the amino acid level) in the active site of reverse transcriptase or integrase proteins to prevent reverse transcription or integration. Rev-independent fragments of the gag gene are described in U.S. patent application Ser. No. 07/858,747, filed Mar. 27, 1992, and also in related U.S. Pat. Nos. 5,972,596 and 5,965,726, which are incorporated by reference herein.

Detailed Description Text - DETX (62):

In the packaging construct shown in FIG. 5, "CMV" denotes the cytomegalovirus promoter, "Gag" denotes the gag gene, which generates components of the virion core, "Pro" denotes "protease" "RT" denotes "reverse transcriptase," "Int" denotes "integrase" and "BGH poly (A)" denotes the bovine growth hormone polyadenylation signal. The protease, reverse transcriptase, and integrase genes comprise the "pol" gene. In transfer construct 1, "LTR" denotes the HIV "long terminal repeat", which contains a HIV promoter; "mSD" denotes "mutated splice donor site," which is present in the construct so that splicing of the RNA transcript does not occur; ".psi." denotes the encapsidation signal; "wGA" denotes part of the wild-type gag gene which contains sequences believed to be necessary for encapsidation; "X" indicates that the ATG codon of the partial gag gene sequence is mutated so that translation of this gene does not occur; "CMV" denotes the cytomegalovirus promoter and luciferase is used as a reporter gene. Luciferase can be replaced with any gene of interest. Another HIV LTR is present at the 3' end of transfer construct 1. Replacement of this LTR in constructs such as the transfer construct 1, 2, or 3 with a promoter-enhancer deleted HIV LTR leads to inactivation of LTR after integration. Transfer construct 2 is similar to transfer construct 1, the difference being that a mutated part of the gag gene (denoted "mGa") is used instead of the wild-type part of the gag gene. Transfer construct 3 (pm2BCwCNIuci) has different mutations at the 5' splice site and has an intact ATG codon so that translation of part of the mutated gag gene occurs. Transfer construct 3 also has a 5' CMV promoter instead of a 5' LTR promoter. This construct is expressed independent of the presence of HIV Tat protein. The transfer constructs expressed from the LTR promoter are partially dependent on Tat protein. In 293 cells significant expression can be achieved in the absence of Tat. See, e.g., Valentin et al., Proc. Natl Acad. Sci. U S A. 95:8886-91 (1988).

US-PAT-NO: 6638732

DOCUMENT-IDENTIFIER: US 6638732 B1

TITLE: Mutants of Green Fluorescent Protein

DATE-ISSUED: October 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Evans; Krista	Germantown	MD	N/A	N/A

APPL-NO: 09/ 472065

DATE FILED: December 23, 1999

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. application Ser. No. 08/970,762, filed Nov. 14, 1997, now abandoned which claims priority to U.S. Provisional Application No. 60/030,935, filed Nov. 15, 1996, the contents of which are entirely incorporated herein by reference.

US-CL-CURRENT: 435/69.1, 435/252.3, 435/320.1, 435/325, 435/410, 530/350, 536/23.5

ABSTRACT:

The present invention provides mutants of the Green Fluorescent Protein (GFP) of *Aequorea victoria*. Specifically provided by the present invention are nucleic acid molecules encoding mutant GFPs, the mutant GFPs encoded by these nucleic acid molecules, vectors and host cells comprising these nucleic acid molecules, and kits comprising one or more of the above as components. The invention also provides methods for producing these mutant GFPs. The fluorescence of these mutants is observable using fluorescein optics, making the mutant proteins of the present invention available for use in techniques such as fluorescence microscopy and flow cytometry using standard FITC filter sets. In addition, certain of these mutant proteins fluoresce when illuminated by white light, particularly when expressed at high levels in prokaryotic or eukaryotic host cells or when present in solution or in purified form at high concentrations. The mutant GFP sequences and peptides of the present invention are useful in the detection of transfection, in fluorescent labeling of proteins, in construction of fusion proteins allowing examination of intracellular protein expression, biochemistry and trafficking, and in other applications requiring the use of reporter genes.

23 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Brief Summary Text - BSTX (34):

One of the earliest mutation studies of GFP, in which the tyrosine residue at position 66 in the wildtype protein ("wt-GFP") was replaced with a histidine residue, resulted in a mutant protein which fluoresced blue instead of green when excited with ultraviolet (UV) light (Heim, R., et al., Proc. Natl. Acad. Sci. USA 91:1250-1254 (1994)). This mutant protein not only provided a capacity for two distinguishable wavelengths for use in studies comparing independent proteins and gene expression events, but also demonstrated that single point mutations in GFP could induce drastic changes in the photochemistry of the protein. Three other sets of specific point mutations have been shown to increase the excitation and emission maxima of GFP such that they fall well within the range of standard fluorescein optics (Ehrig, T., et al., FEBS Letts. 367:163-166 (1995); Delagrave, S., et al., Bio/Technology 13:151-154 (1995); Heim, R., and Tsien, R., Curr. Biol. 6:178-182 (1996)), thus permitting the use of GFP with standard laboratory fluorescence detection systems. The problem of low quantum yield by wt-GFP has been partially addressed by mutating the serine residue at position 65 to a threonine ("S65T"), either without (Heim, R., et al., Proc. Natl. Acad. Sci. USA 91:12501-12504 (1994)) or with (Cormack, B., et al., Gene 173:33-38 (1996)) a concomitant mutation at position 64, or by mutating other residues in the non-chromophore region (Cramer, A., et al., Nature Biotech. 14:315-319 (1996)). The S65T mutation also appears to improve the rate of fluorophore formation in transfected cells by approximately four-fold over wt-GFP, thus allowing earlier and more sensitive detection of transfection with this mutant than with wt-GFP (Heim, R., et al., Proc. Natl. Acad. Sci. USA 91:1250-1254 (1994)). By combining the S65T mutation with a mutation at position 64 replacing phenylalanine with leucine, approximately 90% of the mutant GFP expressed in bacteria is soluble, thus improving protein purification and yields (Cormack, B., et al., Gene 173:33-38 (1996)). Another series of mutations results in a mutant fusion GFP consisting of linked blue- and green-fluorescing proteins which have proven useful in studies of protein localization, targeting and processing (Heim, R., and Tsien, R. Y., Curr. Biol. 6:178-182 (1996)). Analogously, chimeric constructs comprising GFP linked to other proteins have been used in studies of ion channel expression and function (Marshall, J., et al., Neuron 14:211-215 (1995)), and in organelle targeting studies where they have provided a means for selectively and distinctively labeling the organelles of living cells (Rizzuto et al., Curr. Biol. 6:183-188 (1996)). Finally, by combining the S65T mutation with other mutations throughout the nonchromophore regions of the wt-GFP gene, a "humanized" mutant GFP (SEQ ID NOs:3, 4) has been produced that not only shows a significant increase in fluorescence intensity and rate of fluorophore formation over wt-GFP (via the S65T mutation) but also demonstrates a 22-fold increased expression efficiency in mammalian cells (Evans, K., et al., FOCUS 18(2):40-43 (1996); Zolotukhin, S., et al., J. Virol. 70:4646-4654 (1996)).

This humanization was achieved via 92 base substitutions (in 88 codons) to the wt-GFP gene which were amino acid-conservative and which were made to provide a pattern of codon usage more closely resembling that of mammalian cells, as opposed to the jellyfish codon patterns found in the wt-GFP gene which are less efficiently translated in mammalian cells. A summary of these GFP chromophore mutants is presented in Table 1.

Drawing Description Text - DRTX (2):

FIG. 1 is a depiction of the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of humanized S65T mutant A. victoria Green Fluorescent Protein cDNA (after Zolotukhin, S., et al., J. Virol. 70:4646-4654 (1996)).

Detailed Description Text - DETX (5):

Accordingly, in the present invention, specific mutations are introduced into positions 64 and 65 of the wt-GFP cDNA sequence (SEQ ID NO:3). Alternatively, increased expression of the present mutant GFPs may be obtained by introducing the preferred mutations into a humanized GFP gene such as that described previously (SEQ ID NO:1) (Evans, K., et al., FOCUS 18(2):40-43 (1996); Zolotukhin, S., et al., J. Virol. 70:4646-4654 (1996)).

Detailed Description Text - DETX (36):

As depicted in FIG. 5, pGreenLantern-1 (Life Technologies, Inc., Rockville, Md.; catalogue no.10642) contains the humanized S65T mutant GFP cDNA (FIG. 1; SEQ ID NOs: 1, 2) (Evans, K., et al., FOCUS 18(2):40-43 (1996); Zolotukhin, S., et al., J. Virol. 70:4646-4654 (1996)). This plasmid serves as the source of the GFP DNA sequence used for mutagenesis. As depicted in FIG. 6, pGreenLantern-2 contains a universal sequencing primer downstream of the CMV promoter along with an NsiI site immediately upstream of the CMV promoter allowing excision of the promoter region. It also contains XbaI, XhoI and HindIII sites in place of the 3' NotI site in pGreenLantern-1. A stop codon in the 5' multiple cloning site of pGreenLantern-1 was shifted out of frame to allow possible fusions to GFP in pGreenLantern-2.

US-PAT-NO: 6623922

DOCUMENT-IDENTIFIER: US 6623922 B1

TITLE: Methods for identifying, characterizing, and evolving  
cell-type specific CIS regulatory elements

DATE-ISSUED: September 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kamb; Carl Alexander	Salt Lake City	UT	N/A	N/A
Caponigro; Giordano M.	Salt Lake City	UT	N/A	N/A

APPL-NO: 08/ 800664

DATE FILED: February 14, 1997

US-CL-CURRENT: 435/6, 435/320.1, 435/5, 435/69.1, 435/DIG.17, 435/DIG.23  
, 435/DIG.3, 435/DIG.37, 536/23.1

ABSTRACT:

The invention provides methods for efficient and rapid identification of cis-acting nucleic acid sequences that act in a cell-type specific manner to stimulate or repress the expression of linked genes or other neighboring sequences. The invention also provides methods for evolving novel regulatory sequences by in vitro manipulation of naturally occurring or synthetic cis acting nucleic acid sequences followed by screening and counterscreening steps. Furthermore, the invention provides methods for determining the mechanism by which cell-type specific cis regulatory sequences confer cell-type specific expression. Also provided are diagnostic methods based on the use of cell-type specific cis regulatory sequences.

12 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

----- KWIC -----

Detailed Description Text - DETX (45):

The invention permits identification of novel regulatory elements that involve sequence variants, combinations and permutations of natural promoters, enhancers, negative regulatory sequence elements, and/or synthetic DNA sequences. The methods used to create such non-natural sequences include the following types of manipulations. Sub-library sequences that have a particular



activity are either mutated in vitro by any of several methods known in the art, or rejoined with other natural or non-natural fragments by ligation, or digestion and re-ligation (Ausubel F. M., Brent R., et al., 1996). These new sub-libraries are passed through the same host cells (or different cell types) and the selection and counter selection steps are repeated. The method thus permits the evolution of more desirable properties in a series of steps that involve manipulation of library sequences in vitro followed by selection in vivo. Thus, it is possible to evolve, e.g., a cis sequence that is more completely "off" in one cell type and more active in another.

Detailed Description Text - DETX (67):

pEGFP-C1 (Clontech Laboratories, Palo Alto, Calif.; GenBank accession number U55763) is used as a starting material to construct the parental vector. It contains the GFP coding sequence flanked by a CMV promoter/enhancer on its 5' side, and the SV40 T-Antigen gene polyadenylation signal on the 3' side (FIG. 1). This vector is modified so that upstream of the GFP translational start codon are sequences that either include part of the functional promoter (the TATA box from the CMV promoter, generated by trimming pEGP-C1 to a position -63 base pairs from the translational start codon), or sequences completely missing the promoter (trimmed to -10 base pairs upstream of the GFP start). These two crippled ("dead") expression vectors lack sequences necessary for GFP expression in most mammalian cells. The vector is further engineered so that restriction enzyme recognition sites, useful for inserting library fragments, are introduced at positions -63 and -69.

US-PAT-NO: 6573099

DOCUMENT-IDENTIFIER: US 6573099 B2

TITLE: Genetic constructs for delaying or repressing the  
expression of a target gene

DATE-ISSUED: June 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Graham; Michael Wayne	St. Lucia	N/A	N/A	AU

APPL-NO: 09/ 100812

DATE FILED: June 19, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
AU	PP2492	March 20, 1998

US-CL-CURRENT: 435/455, 424/93.2 , 424/93.21 , 435/320.1 , 435/325 , 514/44  
, 536/24.5

ABSTRACT:

The present invention relates generally to synthetic genes for modifying endogenous gene expression in a cell, tissue or organ of a transgenic organism, in particular a transgenic animal or plant. More particularly, the present invention provides novel synthetic genes and genetic constructs which are capable of repressing delaying or otherwise reducing the expression of an endogenous gene or a target gene in an organism when introduced thereto.

22 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 28

----- KWIC -----

Detailed Description Text - DETX (13):

Generally, a gene of the invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions without affecting its ability to modify target gene expression. Nucleotide insertional derivatives of the synthetic gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in

which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product.

Detailed Description Text - DETX (23):

Alternatively, the structural gene may comprise a nucleotide sequence which does not encode an amino acid sequence or more commonly, comprises one or more open reading frames which encode one or more peptides, oligopeptides or polypeptides which are unrelated at the amino acid sequence level to the amino acid sequence encoded by the target gene. For example, the mRNA product of the structural gene may be inserted into the synthetic gene of the invention so as to alter or disrupt the reading frame of the structural gene and produce one or more frame shift mutations in the translation product thereof relative to the translation product encoded by the target gene, notwithstanding a substantial identity between the structural gene and the target gene on the one hand and the corresponding mRNA products of the structural gene and the target gene on the other hand. Such effects may be produced by introducing one or two nucleotide substitutions or deletions into the structural gene, relative to the target gene sequence or alternatively, by introducing a translation start codon 5'-ATG-3' upstream of any nucleotide in the structural gene which occurs at a particular position in a codon of the corresponding target gene such that the position of said nucleotide in the codon of the structural gene is altered.

Detailed Description Text - DETX (174):

Plasmid pCMVLacI.TYR.OPRSV/1.GFP (FIG. 28) is a dual construct in which the CMV IE promoter drives expression of the lacI gene and the mRNA of the mouse tyrosinase cDNA or a fragment thereof, whilst the OPRSVI promoter drives expression of GFP operably under control of the lacI gene. The construct is designed such that the mouse tyrosinase gene is fused to the 3' untranslated region of the lacI gene via a unique BsaB1 cloning site. This cloning site is located after the stop codon of the lacI coding sequence, but before the SV40 polyadenylation signal. The construct also contains the hygromycin-resistance gene as a selection marker.

US-PAT-NO: 6573098

DOCUMENT-IDENTIFIER: US 6573098 B1

TITLE: Nucleic acid libraries

DATE-ISSUED: June 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer, Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 240307

DATE FILED: January 29, 1999

PARENT-CASE:

This application is a CON of and claims the benefit of 08/621,859 filed Mar. 25, 1996, now U.S. Pat. No. 6,117,679; which is a CIP of 08/564,955 filed Nov. 30, 1995, now U.S. Pat. No. 5,811,238; which is a CIP of 08/537,874 filed Mar. 4, 1996 which is a 371 national phase PCT/US95/02126 filed Feb. 17, 1995, and is now U.S. Pat. No. 5,830,721; which is a CIP of 08/198,431 filed Feb. 17, 1994, now U.S. Pat. No. 5,605,793; the disclosures of which are incorporated by reference.

US-CL-CURRENT: 435/440, 536/23.1 , 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

29 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (91):

The initial small population of the specific nucleic acid sequences having

mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into *E. coli* and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (98):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (103):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (169):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L

cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (175):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernel set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (513):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

Detailed Description Text - DETX (529):

E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD<sub>sub 600</sub>. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and GFP promoters are quite

different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6541197

DOCUMENT-IDENTIFIER: US 6541197 B2

TITLE: Vehicles for stable transfer of green fluorescent  
protein gene and methods of use for same

DATE-ISSUED: April 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Link, Jr.; Charles J.	Des Moines	IA	N/A	N/A
Levy; John P.	West Des Moines	IA	N/A	N/A
Wang; Suming	Des Moines	IA	N/A	N/A
Seregina; Tatiana	West Des Moines	IA	N/A	N/A

APPL-NO: 08/ 786531

DATE FILED: January 21, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of provisional application  
Serial. No. 60/010,371 filed Jan. 22, 1996.

US-CL-CURRENT: 435/5, 435/320.1, 435/325, 435/350, 435/351, 435/352  
, 435/366, 435/4, 435/6

ABSTRACT:

The present invention describes an efficient retroviral or viral based method that allows easy and quick identification of gene transfer in living, transduced mammalian cells. Retroviral and viral vector producer cells were generated containing a gene for an improved humanized red-shifted, Green Fluorescent Protein (hRGFP) which increases the resulting fluorescence yield after excitation. This humanized, red-shifted GFP (hRGFP) gene was cloned into several vectors and transfected into various packaging cell lines to produce vibrant green fluorescence after excitation with blue light at 450-490 nm. These vectors represent a substantial advance over currently available gene transfer marking systems or wild-type GFP marker systems none of which have been stably transfected into cells.

5 Claims, 66 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 55



----- KWIC -----

Detailed Description Text - DETX (32):

The instant invention demonstrates the effectiveness of a humanized, red shifted mutant GFP by retroviral and viral mediated gene transfer into human tumor cells and murine fibroblasts. A few molecular genetics groups have now reported mutations of the wild-type GFP gene which can generate GFP gene products with modified excitation and emission spectra. See for e.g. Heim, R., et al. (1994) Proc Natl Acad Sci. USA 91:12501-12504. The longer wavelength excitation peak (475 nm) of native A. Victoria GFP has lower amplitude than its shorter wavelength excitation peak (470-490 nm) with fluorescence amplitudes from 4-6 fold greater than from the wild-type gene product. Heim, R. (1995) Nature 373:663-664. Interestingly, this mutant also had more rapid formation of the fluorochrome. Id. Furthermore, the mutated, red shifted GFP had its codons modified to usage common in mammals (Dr. Sergei Zolotukhi and Dr. Nicholas Muzyczka, University of Florida, unpublished results). The inventors have evaluated this humanized version of a serine-65 to threonine codon mutant that demonstrates excitation at 490 nm and emissions at 510 nm in current gene transfer experiments. Comparisons between the wild-type GFP and the humanized, serine-65 red shifted mutant (hRGFP) demonstrated substantial improvement in fluorescence expression after either transfection and retroviral mediated GFP gene transfer (Table 1).

Detailed Description Text - DETX (35):

The present invention demonstrates the ability to analyze and easily detect living, retroviral transduced cells without the need for cell fixation or antibodies. This allows for the clinical application of this marker in living tissue by using the appropriate enhancer/promoter or targeted transduction procedure. This may be particularly useful in bone marrow processing, lymphocyte sorting, and other applications requiring the FACS analysis of living cells. Further, since the GFP gene has not been shown to have any cytotoxicity in vitro, it is expected that the GFP gene will be safe for in vivo use. Future directions will lead to a wider range of useful GFP based mutant proteins with well defined fluorophores with characterized excitation and emission spectra, using mammalian codon usage. For example, Heim and colleagues subjected the native GFP sequence to random mutations in bacteria and developed a series of interesting mutants with altered excitation peaks and found that a substitution of Tyrosine-66 to histidine generated a mutant GFP that demonstrated blue fluorescent emission. Heim, R., et al. (1994) Proc Natl Acad Sci, USA 91:12501-12504. This may permit two color separation and analysis of living cells by FACS.

Detailed Description Text - DETX (56):

These results demonstrate the effectiveness of a humanized, red-shifted mutant GFP by retroviral mediated gene transfer into human tumor cells and murine fibroblasts.

Detailed Description Paragraph Table - DETL (2):

TABLE 2 SEQUENCE AND RESTRICTION SITE INFORMATION pHGFP-S65T Humanized **GFP** Vector (SEQ ID NO:1) (GenBank Accession # U43284) Location of features Human cytomegalovirus (CMV) immediate early **promoter**: 152-739 Enhancer region: 229-635 TATA box: 724-730 T7 **promoter**: 784-803 Green fluorescent protein gene (S65T variant) Start **codon** (ATG): 826-828; Stop **codon**: 1543-1545 **GFP** fluorescent chromophore: 1021-1029 SV40 small t antigen intron: 1642-1706 SV40 early mRNA polyadenylation signal Polyadenylation signals: 2312-2317 & 2341-2346 mRNA 3' ends: 2350 & 2362 SV40 origin of replication: 2805-2740 pBR322 plasmid replication origin: 2767-3347 M13 single-strand DNA origin: 3367-3934 **Synthetic supF gene**: 4145-3947 Geneology From To 1549 811 pCDM7 vector backbone (Not I-Hind III) 1 151 Fragment from the Rous Sarcoma Virus (RSV) LTR 152 738 Fragment from Human Cytomegalovirus (CMV) containing the immediate early **promoter** 812 1548 **Synthetic GFP gene** using optimal human codons 817 829 Synthetic Kozak consensus translation initiation sequence 829 831 Additional valine not present in wt **GFP** 1021 1023 S65T **mutation in GFP** chromophore replacing serine 65 with threonine [Heim, R. et al. (1995) Nature 373:663-664] 1565 2174 Fragment from SV40 providing small antigen intron 2175 2415 Fragment from SV40 providing polyadenylation signals 2416 2759 Fragment from SV40 providing origin of replication 2767 3347 Fragment from pBR322 providing origin of replication 3367 3934 Fragment from M13 providing single-stranded DNA origin 3947 4145 **Synthetic supF gene** Propagation in E. coli Suitable host strain: MC1061/P3 Selectable Marker: The supF gene confers resistance to ampicillin (25-40 .mu.g/ml) and tetracycline (7.5-10 .mu.g/ml) to MC1061/P3 due to expression of a tRNA that suppresses amber **mutations** in the ampicillin and tetracycline genes on the P3 plasmid. E. coli replication origin: pBR322 (rop.sup.-) Copy number: = 100-200 Plasmid incompatibility group: pMB1/ColE1

US-PAT-NO: 6540996

DOCUMENT-IDENTIFIER: US 6540996 B1

TITLE: Compound screening methods

DATE-ISSUED: April 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zwaal; Richard	Ghent	N/A	N/A	BE
Groenen; Jose	St-Martens Latem	N/A	N/A	BE
Bogaert; Thierry	Kortrijk	N/A	N/A	BE

APPL-NO: 09/ 549872

DATE FILED: April 14, 2000

PARENT-CASE:

RELATED APPLICATIONS

This application claims priority under Title 35 .sctn.119(e) of U.S. Provisional Application No. 60/129,596, filed Apr. 15, 1999, and entitled COMPOUND SCREENING METHODS and foreign priority benefits under Title 35, U.S.C., .sctn.119(a)-(d) or .sctn.365(a),(b) of foreign patent application nos. GB 9908670.4, filed Apr. 15, 1999, and GB 9912736.7, filed Jun. 1, 1999, the entire contents of which are incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9908670	April 15, 1999
GB	9912736	June 1, 1999

US-CL-CURRENT: 424/93.21, 435/4 , 800/13 , 800/3 , 800/8

ABSTRACT:

The invention provides methods of screening for compounds which affect the activity of a physiologically important calcium pump, the sarco/endoplasmic reticulum Ca.sup.2+ ATPase (SERCA), using the nematode worm *C. elegans*.

19 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Detailed Description Text - DETX (17):

A 5026 bp fragment of the upstream region of the *C. elegans* SERCA gene, starting 5026 bp upstream of the translation initiation codon and continuing up to and including the A of the ATG initiation codon (SEQ ID NO:3), was cloned into the vector pPD95.79 (described in Fire et al. (1990) *Gene*, 93: 189-198) in fusion with a GFP fluorescent protein, resulting in vector pGK10. The cloned fragment can be considered as the promoter region of the *C. elegans* SERCA. The vector was injected into *C. elegans*, using standard methodology well known to persons skilled in the art, and the expression of the GFP was monitored applying standard fluorescent techniques. GFP expression was observed all over the early embryo of the worm, although expression was faint in some tissues. In a later stage of development, from mid-embryo stage, through larval stage to adult stage, strong GFP expression could be observed in all muscle tissue, including the pharyngeal muscles, the body wall muscles, the anal depressor and the anal sphincter. In adults staining was seen in the vulva muscles, the uterine muscles, the spermathecae and the proximal myoepithelial sheath cells of the gonad.

Detailed Description Text - DETX (18):

A construct containing a smaller promoter fragment, including A of the initiating ATG codon and extending 2915 bp upstream (SEQ ID NO:4), fused to a GFP gene was generated by a PstI deletion of the plasmid pGK10. This plasmid was designated pGK13. Transfection of the nematode with pGK13 resulted in the same pattern of GFP expression as was observed with pGK10.

Detailed Description Text - DETX (92):

oGK52 contains T-to-G point mutation compared to pig PLB cDNA so as to introduce a D-to-E amino acid substitution at position 2 of PLB. Since this is the only difference between the human and pig PLB proteins, the resultant polypeptide is the same as the human PLB sequence (NB the point mutated cDNA does not have the same sequence as the human PLB cDNA but encodes a protein having identical amino acid sequence to human PLB, hence it is referred to as a humanized pig PLB cDNA).

Detailed Description Text - DETX (155):

Sequence Listing SEQ ID NO:1 is the nucleic acid sequence of a 732 bp EcoRI-HindIII fragment of *C. elegans* SERCA exon 5. This fragment was cloned into pGEM3 for use in RNA inhibition experiments. SEQ ID NO:2 is the nucleic acid sequence of a 11207 bp SpeI-MluI fragment of cosmid K11D9. This fragment contains the complete *C. elegans* SERCA gene with 5631 bp of upstream sequence, the entire coding region and 1088 bp of downstream sequence. The fragment was cloned into pUC18 to give plasmid pGK7. SEQ ID NO:3 is the nucleic acid sequence of a 5026 bp fragment of the upstream region of *C. elegans* SERCA, up to and including A of the initiating ATG. This fragment was cloned into pPD95.79, in fusion with GFP, to give plasmid pGK10. SEQ ID NO:4 is the nucleic acid sequence of a 2915 bp fragment of the upstream region of *C. elegans* SERCA, as found in plasmid pGK13. SEQ ID NO:5 is the nucleic acid

sequence of a 6612 bp fragment of the *C. elegans* SERCA gene containing 5637 bp of upstream sequence and ending in exon 4, as cloned in pPD95.75, resulting in pGK12. SEQ ID NO:6 is the nucleic acid sequence of the long isoform of the *C. elegans* SERCA cDNA. SEQ ID NO:7 is the nucleic acid sequence of the pig SERCA2a cDNA. SEQ ID NO:8 is the nucleic acid sequence of the human SERCA2a cDNA. SEQ ID NO:9 is the nucleic acid sequence of the pig phospholamban cDNA. SEQ ID NO:10 is the nucleic acid sequence of the *C. elegans* myo-2 promoter. SEQ ID NO:11 is the nucleic acid sequence of the *C. elegans* myo-3 promoter. SEQ ID NO:12 is the nucleic acid sequence of the *C. elegans* vulval muscle enhancer. This is an enhancer element from *ceh-24* that directs gene expression in the vulval muscles (Harfe and Fire, 1998, Developmental 125: 421-429) SEQ ID NO:13 is the nucleic acid sequence of humanized pig PLB cDNA. SEQ ID NO:14 is the amino acid sequence of pig PLB. SEQ ID NO:15 is the amino acid sequence of human PLB and humanized pig PLB. SEQ ID NO:16 is the nucleotide sequence of a genomic fragment of *C. elegans* SERCA covered by primers SERCA P4 and SERCA P8. SEQ ID Nos: 17-38 are primers used in the accompanying Examples. SEQ ID NO:39 is an amino acid sequence insertion of a mutant *C. elegans* SERCA ATPase

US-PAT-NO: 6531123

DOCUMENT-IDENTIFIER: US 6531123 B1

TITLE: Lentiviral vectors

DATE-ISSUED: March 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chang; Lung-Ji	Gainesville	FL	32606	N/A

APPL-NO: 09/ 318138

DATE FILED: May 25, 1999

PARENT-CASE:

This application is a continuation-in-part of Ser. No. 08/935,312, filed Sep. 22, 1997, now U.S. Pat. No. 6,207,455, which is incorporated by reference to the extent that it does not directly conflict with the teachings of the present application. That application is a continuation-in-part of Ser. No. 08/848,760, filed May 1, 1997 now U.S. Pat. No. 6,248,721.

This application is also a nonprovisional of Ser. No. 60/086,635, filed May 26, 1998, which likewise is incorporated by reference.

US-CL-CURRENT: 424/93.2, 424/93.1, 424/93.6, 435/235.1, 435/320.1, 435/325, 435/366, 435/455, 435/456, 435/457, 435/5, 435/6, 536/23.1, 536/23.72, 536/24.1

ABSTRACT:

The present invention contemplates novel lentiviral vectors which exhibit strong promoter activity in human and other cells. Vectors are provided which are packaged efficiently in packaging cells and cell lines to generate high titer recombinant virus stocks. The present invention further relates to HIV vaccines and compositions for gene therapy. In particular, the present invention provides attenuated replication-competent HIV vaccines and replication defective HIV vectors.

37 Claims, 31 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 26

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Detailed Description Text - DETX (445):

To engineer a packaging signal for the construction of HIV-1 transducing vectors (TV), an artificial HIV-1.psi. sequence using four synthetic oligonucleotides was synthesized, which comprised sequences between the PBS and the gag AUG (referred to as ".psi.100" or "PAK100") and sequences extending into the gag ORF (referred to as ".psi.140" or "PAK140"). These synthetic HIV-1.psi. sequences contained a mutated SD site (three nucleotides changed in PAK100 and PAK140, GAGTA.fwdarw.CATTTC) and a mutated gag AUG (HindIII and BamHI sites inserted upstream of gag AUG in both; PAK100 stopped just upstream of gag AUG; PAK 140 changed gag AUG to UAG and second codon from GGT to GCC) to avoid possible adverse effects in gene expression. PAK100 and PAK140 both started at nt 690 of provirus, i.e., 5' base of U3=1. The synthetic .psi. signals were cloned into the pTV.psi. vector as shown in FIG. 8, which is comprised of two recombinant LTRs ("dl.kB-CMV/HIV-TAR"), the PBS and 5' leader sequences, an SV40-driven neo resistance gene, and the 3' PPT.

Detailed Description Text - DETX (606):

3. Generation of different DTV-CFTR HIV vectors. Although the study of pTV.DELTA.CMV-nlacZ showed that the internal CMV promoter is a strong promoter after HIV vector transduction, some vector constructs, such as those carrying reporter genes such as GFP or placenta alkaline phosphatase (PLAP) exhibited undetectable amount of gene products in our preliminary studies. Therefore, the optimal CFTR HIV vector will have to be empirically established. pTV constructs containing either CMV or human elongation factor 1a internal promoter will be used to generate CFTR vectors. The upstream HIV major splice donor site and the gag AUG initiation codon have both been deleted without affecting vector titers. These different CFTR HIV vector constructs will be generated and used to transduce IB3-1 epithelial cells. The expression of apical CFTR will be immunostained with a monoclonal antibody MATG1031[Demolombe, 1996 #3706], specific to the first extracellular loop sequence of the CFTR protein which is absent in IB3-1 cells. The level of expression will be determined under a confocal microscope.

US-PAT-NO: 6518065

DOCUMENT-IDENTIFIER: US 6518065 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: February 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer; Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 696313

DATE FILED: October 25, 2000

PARENT-CASE:

This application is a continuation of and claims the benefit of U.S. application Ser. No. 09/239,395 filed Jan. 28, 1999, which is a continuation of Ser. No. 08/621,859, filed Mar. 25, 1996 (now U.S. Pat. No. 6,117,679), which is a CIP of U.S. Ser. No. 08/564,955, filed Nov. 30, 1995 (now U.S. Pat. No. 5,811,238), which is a CIP of PCT/US/95/02126, filed Feb. 17, 1995 (which entered the U.S. National Phase as U.S. Ser. No. 08/537,874, now U.S. Pat. No. 5,830,721) which is a CIP of Ser. No. 08/198,431, filed Feb. 17, 1994 (now U.S. Pat. No. 5,605,793), the disclosure of which is incorporated by reference for all purposes.

US-CL-CURRENT: 435/440, 435/6 , 435/91.2 , 536/23.1 , 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

45 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

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Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin 1, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment

of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed **mutagenesis**. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR **mutagenesis** has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed **mutants** (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical **mutagenesis** (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv **mutants**. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region **sequences by randomizing the sequence in a synthetic** CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (175):

For generating diverse variable segments, a collection of **synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence** kernel set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by **mutating** the CDR(s) with site-directed **mutagenesis**, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (474):

A **synthetic gene** was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled **mutant** showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the **mutant** protein(s) were soluble and active. The three amino acid **mutations** thus guide the **mutant** protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous **mutant** variants rapidly and efficiently.

Detailed Description Text - DETX (490):

E. coli expressing the synthetic **GFP** construct ('wt') with altered **codon** usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed

at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and **GFP promoters** are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6514757

DOCUMENT-IDENTIFIER: US 6514757 B1

TITLE: Nodavirus-like DNA expression vector and uses thereof

DATE-ISSUED: February 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ball; Laurence Andrew	Birmingham	AL	N/A	N/A
Johnson; Kyle Leslie	Birmingham	AL	N/A	N/A
Johnson; Karyn Nicole	Birmingham	AL	N/A	N/A
Price; B. Duane	Mountain Brook	AL	N/A	N/A

APPL-NO: 09/ 595346

DATE FILED: June 15, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This non-provisional patent application claims benefit of provisional patent application U.S. Ser. No. 60/139,120, filed Jun. 14, 1999, now abandoned

US-CL-CURRENT: 435/325, 435/320.1 , 536/23.1 , 536/23.72

ABSTRACT:

The present invention describes the production of a nodavirus-based DNA vector that drives abundant expression of foreign genes in a wide variety of cell types. The DNA plasmid is initially transcribed by a host-cell RNA polymerase to produce primary transcripts from which a nodaviral RNA-dependent RNA polymerase (RNA replicase) is translated. These primary transcripts are then amplified by the RNA replicase in an autonomous, cytoplasmic RNA replication. Such a vector is a useful addition to the current arsenal of expression vectors, and well suited to laboratory-scale and larger-scale expression of transcripts and/or proteins in eukaryotic cells.

3 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Detailed Description Text - DETX (23):

A "heterologous" region or gene of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. In another example, the coding sequence itself may not be found in nature (e.g., a cDNA, in which the genomic coding sequence contains introns, or synthetic sequences having codons different from the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

Detailed Description Text - DETX (62):

The next step is to harness RNA replication to drive the amplification of foreign mRNAs. To this end, the tandem delivery plasmid (FIG. 5) is modified by inserting a heterologous gene, in this case for experimental purposes, a reporter gene, as the 5'-proximal open reading frame in the RNA2 sequence (FIG. 6). Genes encoding firefly luciferase, lacZ, G418 resistance or the green fluorescent protein (GFP) are introduced into the downstream RNA2 replicon such that their open reading frames are flanked by the RNA2 cis-acting signals and positioned to initiate translation at the first AUG codon of the chimeric RNA2. To provide a versatile eukaryotic vector for general use, the insertion site for the reporter gene is flanked by several unique restriction sites for convenient cloning of other target genes, i.e., a multiple cloning site (MCS). The above-mentioned reporter genes were chosen because they can be readily assayed over a wide range of expression levels. For experimental controls, conventional monocistronic plasmids are also constructed that lack the RNA replicase gene but contain the same reporter genes flanked by the RNA2 cis-acting signals, placed directly downstream of an RNA pol II promoter. The levels and duration of reporter gene expression from the RNA replicon plasmids are compared with those from the control plasmids that rely solely on DNA-templated transcription for mRNA synthesis.

US-PAT-NO: 6506603

DOCUMENT-IDENTIFIER: US 6506603 B1

TITLE: Shuffling polynucleotides by incomplete extension

DATE-ISSUED: January 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer; Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 717391

DATE FILED: November 18, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of Ser. No. 09/075,511, now U.S. Pat. No. 6,165,793, filed May 8, 1998, which is a continuation of Ser. No. 08/621,859 filed Mar. 25, 1996 now U.S. Pat. No. 6,117,679; which is a CIP of Ser. No. 08/564,955 filed Nov. 30, 1995 now U.S. Pat. No. 5,811,838; which is a CIP of PCT/US95/02126 filed Feb. 17, 1995 which is a CIP of Ser. No. 08/198,431 filed Feb. 17, 1994 now U.S. Pat. No. 5,605,793.

US-CL-CURRENT: 435/440, 435/6, 536/23.1, 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

66 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random,

pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (509):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

Detailed Description Text - DETX (525):

E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present



in the 'Clontech' construct, the expression vectors and **GFP promoters** are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6506602

DOCUMENT-IDENTIFIER: US 6506602 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: January 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer, Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 696322

DATE FILED: October 25, 2000

PARENT-CASE:

This application is a continuation of and claims the benefit of U.S. application Ser. Nos. 09/239,395 filed Jan. 28, 1999, now abandoned which is a continuation of Ser. 08/621,859, filed Mar. 25, 1996 (now U.S. Pat. No. 6,117,689), the disclosures of which are incorporated by reference for all purposes.

US-CL-CURRENT: 435/440, 435/6, 536/23.1, 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

71 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be

created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into *E. coli* and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions

(CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) *Biotechniques* 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) *J. Biol. Chem.* 269: 9533). Riechmann et al. (1993) *Biochemistry* 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (474):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the *E. coli* whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest *E. coli* colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in *E. coli* was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in *E. coli* most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

Detailed Description Text - DETX (490):

*E. coli* expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and GFP promoters are quite different. The cause of the improved fluorescence signal is not enhanced

expression level, it is improved protein performance.

US-PAT-NO: 6468745

DOCUMENT-IDENTIFIER: US 6468745 B1

TITLE: Method for expressing a library of nucleic acid sequence  
variants and selecting desired traits

DATE-ISSUED: October 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fitzmaurice; Wayne P.	Vacaville	CA	N/A	N/A
Lindbo; John A.	Vacaville	CA	N/A	N/A
Padgett; Hal S.	Vacaville	CA	N/A	N/A
Pogue; Gregory P.	Vacaville	CA	N/A	N/A

APPL-NO: 09/ 359304

DATE FILED: July 21, 1999

PARENT-CASE:

This application is a Continuation-In-Part application of U.S. patent application Ser. No. 09/232,170, filed on Jan. 15, 1999, now abandoned, which is a Continuation-In-Part application of U.S. patent application Ser. No. 09/008,186, filed on Jan. 16, 1998.

US-CL-CURRENT: 435/6, 435/235.1, 435/410, 435/440, 435/441, 435/446  
, 435/468, 435/5, 435/69.1, 435/91.4, 536/23.1

ABSTRACT:

The present invention relates to a method for using viral vectors to bear populations of sequence variants and using plant hosts to select the sequences that exhibit the desired traits.

25 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (78):

The RPR method offers several advantages. First, single-stranded polynucleotide templates without an intermediate step of synthesizing the complete second strand may be used in RPR. Potential mutations and/or crossovers may be introduced at the DNA level from single- or double-stranded DNA template by using DNA polymerases, or directly from mRNA by using

RNA-dependent DNA polymerases. Second, DNA shuffling requires fragmentation of the double-stranded DNA template by nucleases, e.g. Dnase I. These nucleases need to be removed completely before the fragments can be reassembled into full length sequences. Sequence reassembly is generally easier with the RPR technique, which employs random priming synthesis to obtain the short DNA fragments. Furthermore, since Dnase I hydrolyzes double-stranded DNA preferentially at sites adjacent to pyrimidine nucleotides, its use in template digestion may introduce a sequence bias into the recombination. Third, the synthetic random primers are uniform in their length and lack sequence bias. The sequence heterogeneity allows them to form hybrids with the template DNA stands at many positions, so that, at least in principle, every nucleotide of the template should be copied or mutated at a similar frequency during extension. The random distribution of the short, nascent DNA fragments along the templates and the random distribution of point mutations within each nascent DNA fragment should guarantee the randomness of crossovers and mutations in the full length progeny genes. Fourth, the random-priming DNA synthesis is independent of the length of the DNA template. Fifth, since the template polynucleotide serves solely as the template for the synthesis of nascent, single-stranded DNA, 10-20 times less template DNA is needed as compared to DNA shuffling.

#### Detailed Description Text - DETX (6):

The first stage of this experiment is the construction of a vector into which shuffled DNA fragments may be reintroduced. The polymerase chain reaction (PCR) was used to amplify a DNA fragment from the TMV vector p30B comprising the T7 promoter, 5' NTR, and the reading frames for the 126 and 183 kDa replicase proteins. The 5' primer covered the T7 promoter and initial bases of the TMV genome while the second primer modified the context surrounding the start codon for the 30 kDa MP of TMV. This allowed DNA fragments to be ligated into the modified vector, designated 30B GFP d30K, as AvrII, PaeI restriction endonuclease digested fragments.

US-PAT-NO: 6432701

DOCUMENT-IDENTIFIER: US 6432701 B1

TITLE: Derived tyrosine hydroxylase gene expression system

DATE-ISSUED: August 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mallet; Jacques	Paris	N/A	N/A	FR
Meloni; Rolando	Paris	N/A	N/A	FR
Ravassard; Philippe	Paris	N/A	N/A	FR
Treilhou; Fabienne	Gif sur Yvette	N/A	N/A	FR

APPL-NO: 09/ 171162

DATE FILED: October 14, 1998

PARENT-CASE:

This application was filed under 35 U.S.C. .sectn.371 and is the National Stage of International Application No. PCT/FR97/00636, filed Apr. 10, 1997.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
FR	96 05223	April 25, 1996

PCT-DATA:

APPL-NO: PCT/FR97/00636  
DATE-FILED: April 10, 1997  
PUB-NO: WO97/40172  
PUB-DATE: Oct 30, 1997  
371-DATE: Oct 14, 1998  
102(E)-DATE: Oct 14, 1998

US-CL-CURRENT: 435/320.1, 536/24.1

ABSTRACT:

The invention discloses a new system for gene expression. The system is based in particular on the use of derived sequences of the first intron of the tyrosine hydroxylase gene having transcription enhancing properties. The system is particularly useful in the production of proteins in vitro, ex vivo or in vivo, particularly in gene therapy applications.

27 Claims, 3 Drawing figures

Exemplary Claim Number: 1



----- KWIC -----

Brief Summary Text - BSTX (2):

Gene and cell therapies consist in correcting a deficiency or an abnormality (mutation, aberrant expression, and the like) or in providing for the expression of a protein of therapeutic interest by introducing genetic information into the cell or organ affected. This genetic information may be introduced either ex vivo into a cell extracted from the organ, the modified cell then being reintroduced into the body (cell therapy), or directly in vivo into the appropriate tissue (gene therapy). Different techniques exist for performing gene transfer, including various techniques of transfection involving natural or synthetic chemical or biochemical vectors such as complexes of DNA and DEAE-dextran (Pagano et al., J. Virol. 1 (1967) 891), of DNA and nuclear proteins (Kaneda et al., Science 243 (1989) 375), and of DNA and lipids (Felgner et al., PNAS 84 (1987) 7413), the use of liposomes (Fraleay et al., J. Biol. Chem. 255 (1980) 10431), cationic lipids, and the like. Another technique is based on the use of viruses as vectors for gene transfer. In this connection, different viruses have been tested for their capacity to infect certain cell populations, and especially retroviruses (RSV, HMS, MMS, and the like), the HSV virus, adeno-associated viruses and adenoviruses. One of the difficulties in developing these gene and cell therapies lies, however, in the efficacy of the treatment. In particular, it is important to be able to obtain a strong expression of the gene of interest in order to improve the therapeutic effect. The same type of problem arises in the case of methods for the production of recombinant proteins.

Brief Summary Text - BSTX (16):

The promoter is advantageously chosen from promoters which are functional in mammalian, preferably human, cells. The promoter in question can be, in particular, one that permits the expression of a nucleic acid in a hyperproliferative cell (cancer cell, restenosis and the like). In this connection, different promoters may be used, such as the p53 gene promoter. A further option comprises regions of different origin (responsible for the expression of other proteins, or even synthetic sequences). Thus, it is possible to use any promoter or derived sequence that stimulates or represses the transcription of a gene, specifically or otherwise, inducibly or otherwise, strongly or weakly. The promoter sequences of eukaryotic or viral genes may be mentioned in particular. Possible promoter sequences are, for example, ones originating from the genome of the target cell. Among eukaryotic promoters, it is possible to use, in particular, ubiquitous promoters (promoter of the HPRT, PGK,  $\alpha$ -actin, tubulin, and the like, genes), promoters of intermediate filaments (promoter of the GFAP, desmin, vimentin, neurofilament, keratin, and the like, genes), promoters of therapeutic genes (for example the promoter of the MDR, CFTR, factor VIII, ApoAI, ApoAII, albumin, thymidine kinase, and the like, genes), tissue-specific promoters (promoter of the pyruvate kinase, villin, intestinal fatty acid binding protein or smooth muscle  $\alpha$ -actin gene, neuronal specific enolase promoter (Forss-Petter et al., Neuron 5 (1990) 187);

and the like), of the promoter generating the V1 form of the mRNA of VACHT (acetylcholine transporter: Cervini et al., J. Biol. Chem. 270 (1995) 24654) or alternatively promoters that respond to a stimulus (steroid hormone receptor, retinoic acid receptor, and the like). Similarly, the promoter sequences may be ones originating from the genome of a virus, such as, for example, the promoters of the adenovirus E1A and MLP genes, the CMV early promoter or alternatively the RSV or MMTV LTR promoter, the herpesvirus TK gene promoter, and the like. In addition, these promoter regions may be modified by adding or deleting sequences. To this end, the promoter uses can be a "minimal" promoter, that is to say a reduced promoter whose activity depends essentially on the presence of a transactivator such as an enhancer of the invention. As a result, in the absence of the enhancer the activity of the promoter is reduced and the gene is not expressed or is expressed to only a small extent. In contrast, in the presence of the enhancer, the activity of the minimal promoter is strong and the expression of the gene of interest substantial. The minimal promoter generally consists of a TATA or INR box. These elements are, in effect, the minimum elements necessary for the expression of a gene in the presence of a transactivator. It advantageously comprises less than 200 bp, including the TATA or INR region. The minimal promoter may be prepared from any promoter by genetic modification. As a particular example of a candidate promoter, the thymidine kinase gene promoter may be mentioned. Advantageous results have, more precisely, been obtained with a minimal promoter derived from the herpes simplex type I thymidine kinase (TK) gene promoter composed of nucleotides -109 to +52, or -37 to +19. The minimal promoter may also be derived from human CMV. In particular, it can consist of the fragment lying between nucleotides -53 and +75 or -31 and +75 of CMV (+1 corresponding to the ATG codon). Any conventional promoter may however be used, such as, for example, the promoter of the genes coding for chloramphenicol acetyltransferase, .beta.-galactosidase or alternatively luciferase.

US-PAT-NO: 6413774

DOCUMENT-IDENTIFIER: US 6413774 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: July 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer; Willem P. C.	Los Gatos	CA	N/A	N/A
Cramieri; Andreas M.	Mountain View	CA	N/A	N/A

APPL-NO: 09/ 240734

DATE FILED: January 29, 1999

PARENT-CASE:

This application is a continuation of and claims the benefit of U.S. application Ser. Nos. 08/621,859, filed Mar. 25, 1996 (Now U.S. Pat. No. 6,117,679), which is a continuation-in-part of USSN 08/564,955, filed Nov. 30, 1995 (Now U.S. Pat. No. 5,811,238), which is a continuation-in-part of PCT/US95/02126, filed Feb. 17, 1995, designating the United States (and which entered the U.S. National Phase as USSN 08/537,874, Now issued as U.S. Pat. No. 5,830,721), the disclosures of which are incorporated by reference.

US-CL-CURRENT: 435/440, 435/6 , 536/23.1 , 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

36 Claims, 35 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into *E. coli* and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin 1, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (167):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the

V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed **mutagenesis**. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR **mutagenesis** has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed **mutants** (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical **mutagenesis** (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv **mutants**. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region **sequences by randomizing the sequence in a synthetic** CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (173):

For generating diverse variable segments, a collection of **synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence** kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by **mutating** the CDR(s) with site-directed **mutagenesis**, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (557):

A **synthetic gene** was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled **mutant** showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the **mutant** protein(s) were soluble and active. The three amino acid **mutations** thus guide the **mutant** protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous **mutant** variants rapidly and efficiently.

Detailed Description Text - DETX (573):

E. coli expressing the synthetic **GFP** construct ('wt') with altered **codon** usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of

poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and **GFP promoters** are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6395547

DOCUMENT-IDENTIFIER: US 6395547 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: May 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer; Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 619550

DATE FILED: July 19, 2000

PARENT-CASE:

This application is a DIV of Ser. No. 09/239,395 filed Jan. 28, 1999, which is a CON of Ser. No. 08/621,859 filed Mar. 25, 1996, now U.S. Pat. No. 6,117,679; which is a CIP of Ser. No. 08/564,955 filed Nov. 30, 1995, now U.S. Pat. No. 5,811,238; which is a CIP of Ser. No. 08/537,874 filed Mar. 4, 1996, now U.S. Pat. No. 5,830,721; which is the national phase of PCT/US95/02126 filed Feb. 17, 1995; which is a CIP of Ser. No. 08/198,431 filed Feb. 17, 1994, now U.S. Pat. No. 5,605,793 the disclosure of which is incorporated by reference for all purposes.

US-CL-CURRENT: 435/440, 435/6, 536/23.1, 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

42 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the By original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin 1, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression ID of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the



V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed **mutagenesis**. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR **mutagenesis** has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed **mutants** (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical **mutagenesis** (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv **mutants**. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region **sequences by randomizing the sequence in a synthetic** CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of **synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence** kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by **mutating** the CDR(s) with site-directed **mutagenesis**, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (585):

A **synthetic gene** was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies; for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled **mutant** showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the **mutant** protein(s) were soluble and active. The three amino acid **mutations** thus guide the **mutant** protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous **mutant** variants rapidly and efficiently.

Detailed Description Text - DETX (601):

E. coli expressing the synthetic **GFP** construct ('wt') with altered **codon** usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of

poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and **GFP promoters** are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6384207

DOCUMENT-IDENTIFIER: US 6384207 B1

TITLE: Regulatory sequences for transgenic plants

DATE-ISSUED: May 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ainley; Michael	Carmel	IN	N/A	N/A
Armstrong; Katherine	Zionsville	IN	N/A	N/A
Belmar; Scott	Indianapolis	IN	N/A	N/A
Folkerts; Otto	Guilford	CT	N/A	N/A
Hopkins; Nicole	Indianapolis	IN	N/A	N/A
Menke; Michael A.	Indianapolis	IN	N/A	N/A
Pareddy; Dayakar	Carmel	IN	N/A	N/A
Petolino; Joseph F.	Zionsville	IN	N/A	N/A
Smith; Kelley	Lebanon	IN	N/A	N/A
Woosley; Aaron	Fishers	IN	N/A	N/A

APPL-NO: 09/ 097319

DATE FILED: June 12, 1998

PARENT-CASE:

RELATED APPLICATIONS

This application claims the priority of U.S. patent application Ser. No. 60/049,752, filed Jun. 12, 1997.

US-CL-CURRENT: 536/24.1, 435/320.1, 435/410, 435/419, 536/23.1, 800/298

ABSTRACT:

Regulatory sequences derived from a maize root preferential cationic peroxidase gene (Per5), including the promoter, introns, and the 3' untranslated region, are useful to control expression of recombinant genes in plants.

4 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (111):

As used in the present application, the term "substantial sequence homology" is used to indicate that a nucleotide sequence (in the case of DNA or RNA) or an amino acid sequence (in the case of a protein or polypeptide) exhibits substantial, functional or structural equivalence with another nucleotide or amino acid sequence. Any functional or structural differences between sequences having substantial sequence homology will be de minimis; that is they will not affect the ability of the sequence to function as indicated in the present application. For example, a sequence which has substantial sequence homology with a DNA sequence disclosed to be a root-preferential promoter will be able to direct the root-preferential expression of an associated DNA sequence. Sequences that have substantial sequence homology with the sequences disclosed herein are usually variants of the disclosed sequence, such as mutations, but may also be synthetic sequences.

#### Detailed Description Text - DETX (231):

Production of the GUS protein from genes controlled by different promoter versions was often compared relative to an internal control gene that produced firefly luciferase. DeWet et al (1987). A plasmid (pT3/T7-1 LUC) containing the luciferase (LUC) coding region was purchased from CLONTECH (Palo Alto, Calif.), and the coding region was modified at its 5' and 3' ends by standard methods. Briefly, the sequences surrounding the translational start (ATG) codon were modified to include an NcoI site (CCATGG) and an alanine codon (GCA) at the second position. At the 3' end, an Ssp I recognition site positioned 42 bp downstream of the Stop codon of the luciferase coding region was made blunt ended with T4 DNA polymerase, and ligated to synthetic oligonucleotide linkers encoding the BglII recognition sequence. These modifications permit the isolation of the intact luciferase coding region on a 1702 bp fragment following digestion by NcoI and BglII. This fragment was used to replace the GUS gene of plasmid pDAB305 (see Example 24E, step 5), such that the luciferase coding region was expressed from the enhanced 35S promoter, resulting in plasmid pDeLux. The 5' untranslated leader of the primary transcript includes the modified MSV leader/Adh intron sequence.

#### Detailed Description Paragraph Table - DETL (1):

TABLE 1 Features of pDAB 406 nt (SEQ ID NO 8) Features 1-6 Apal site 7-24 multiple cloning site (NheI, KpnI, SmaI) 25-30 SalI site 32-1840 E. coli uidA reporter gene encoding the beta-glucuronidase protein (GUS) from pKA882 and TGA stop codon 1841-1883 3' untranslated region from pBI221 1894-1899 SstI site 1900-2168 nopaline synthetase 3' polyA sequence (nos 3'UTR) 2174-2179 HindIII site 2180-2185 BglII site 2186-2932 a modified CaMV 35S promoter 2195-2446 MCASTRAS nt 7093-7344 2455-2801 MCASTRAS nt 7093-7439 2814-2932 Synthetic Maize Streak Virus (MSV) untranslated leader containing the maize Adh1 intron 1 2933-2938 BglII/BclI junction 2933-3023 Adh1.S nt 269-359 MZEAHD1.S 3024-3141 Adh1.S nt 704-821 MZEAHD1.S 3146-3151 BamHI/BglII junction 3150-3187 synthetic MSV leader containing the maize Adh1 intron 1 3188-3193 NcoI 3190-4842 internal reference gene composed of the firefly luciferase gene (Lux) 4907-5165 nopaline synthetase 3' polyA sequence (nos 3'UTR) 5172-5177 BglII site 5178-5183 NdeI site 5186-5191 SstI site 5195-5672 nt 6972-6495 MCASTRAS (CaMV 35S promoter) 5680-6034 nt 7089-7443 MCASTRAS (CaMV 35S promoter) 6042-7021 Tn5 nt 1539-2518; mutated 2X 6054-6848

a selectable marker gene composed of the bacterial NPTII gene encoding neomycin phosphotransferase which provides resistance to the antibiotics kanamycin, neomycin and G418 7022-7726 3' UTR of ORF26 gene *Agrobacterium tumifaciens* Ti plasmid (pTi 15955, nt 22438 to 21726) 7727-7732 NdeI site 7733-7914 pUC19 nt 1-182, reverse complement 7915-10148 nt 453 to 2686 pUC19, reverse complement 10149-10160 multiple cloning site, HindIII, SstI

US-PAT-NO: 6383794

DOCUMENT-IDENTIFIER: US 6383794 B1

TITLE: Methods of producing high titer recombinant  
adeno-associated virus

DATE-ISSUED: May 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mountz; John D.	Birmingham	AL	N/A	N/A
Zhang; Huang-Ge	Birmingham	AL	N/A	N/A

APPL-NO: 09/ 379841

DATE FILED: August 24, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application claims benefit of priority under 35 USC .sctn.119(e) of U.S. provisional application Ser. No. 60/097,666 filed Aug. 24, 1998.

US-CL-CURRENT: 435/235.1, 435/320.1

ABSTRACT:

The present invention discloses a method of producing large-scale recombinant adeno-associated virus stocks by infection of cells with at least one recombinant adenovirus vector(s). The vector(s) encode a therapeutic gene flanked by the terminal repeat ends of adeno-associated virus and the adeno-associated virus rep and cap genes. Transfection with two recombinant adenovirus vector(s) instead of two plasmids plus adenovirus results in the large scale, high titer production of recombinant adeno-associated virus with little to no contaminating adenovirus present.

11 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

----- KWIC -----

Detailed Description Text - DETX (28):

A "heterologous" region of the DNA construct is an identifiable segment of

DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. In another example, the coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

Detailed Description Text - DETX (73):

Attenuation of rep translation efficiency is carried out by changing the rep translation start codon from ATG to ACG in pShuttleAAV (FIG. 1), and then construction of the recombinant AdAAV expressing the rep gene with the ACG start codon (FIG. 8A). This codon change was shown to attenuate the translation efficiency of the rep gene. It is also possible to enhance the AAV capsid protein expression by replacing the p40 promoter with the chicken .beta.-actin promoter and the HCMV enhancer (which utilizes a CAG start codon) (FIG. 8A). Co-infection of this virus (FIG. 8A) with AdrAAV-GFP (FIG. 8B) results in high-titer production of rAAV-GFP. The production of rAAV-GFP is then evaluated under optimized viral dose conditions.

US-PAT-NO: 6372497

DOCUMENT-IDENTIFIER: US 6372497 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: April 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer, Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 590774

DATE FILED: June 8, 2000

PARENT-CASE:

This application is a CON of Ser. No. 08/621,859 filed Mar. 25, 1996, now U.S. Pat. No. 6,117,679; which is a CIP of Ser. No. 08/564,955 filed Nov. 30, 1995, now U.S. Pat. No. 5,811,238; which is a CIP of Ser. No. 08/537,874 filed Mar. 4, 1996, now U.S. Pat. No. 5,830,721; which is the national phase of PCT/US95/02126 filed Feb. 17, 1995; which is a CIP of Ser. No. 08/198,431 filed Feb. 17, 1994, now U.S. Pat. No. 5,605,793; the disclosures of which are herein incorporated by reference for all purposes.

US-CL-CURRENT: 435/440, 536/23.1 , 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

37 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (90):



The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random,

pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernel set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (535):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

Detailed Description Text - DETX (551):

E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present

in the 'Clontech' construct, the expression vectors and GFP promoters are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6361998

DOCUMENT-IDENTIFIER: US 6361998 B1

\*\*See image for Certificate of Correction\*\*

TITLE: Efficient culture of stem cells for the production of hemoglobin

DATE-ISSUED: March 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bell; David N.	Oakville	N/A	N/A CA	
Matthews; Kathryn Emma	Toronto		N/A N/A CA	
Mueller; Susan G.	Milton	N/A	N/A CA	

APPL-NO: 09/ 339838

DATE FILED: June 25, 1999

US-CL-CURRENT: 435/407, 435/325 , 435/405 , 435/69.1 , 435/70.1 , 530/350 , 536/23.5

ABSTRACT:

The present invention describes a serum-free medium that promotes the growth and differentiation of erythroid cells, cells that are highly transducible by a non-viral method and genes which increase the growth of erythroid cells and decrease their dependency on Epo. This invention can be used in the expansion of hematopoietic stem cells to produce cultures of erythroid cells as a source of erythroid-specific proteins such as hemoglobin. Hematopoietic stem cells are cultured ex vivo in a serum-free culture medium with the addition of IL-3, SCF and EPO. Cells transfected with the gene described in the present invention can be cultured in the serum-free culture medium with decreased dependency on Epo and other cytokines, thereby reducing the cost of the production of hemoglobin.

15 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Detailed Description Text - DETX (67):

Freshly isolated CD34.sup.+ enriched cells, prepared as described in Example 1, were washed in electroporation buffer (EB) (composed of D-PBS plus 11 mM

glucose) and resuspended in EB to a density of between 5.0.times.10.sup.6 and 1.3.times.10.sup.7 cells/ml. Cells were placed on ice for 10 minutes prior to electroporation. Immediately prior to electroporation, supercoiled plasmid DNA (or an equivalent volume of D-PBS) was added to the cell sample to a final concentration of 50 ug/ml. Cells were electroporated by standard means using the conditions described in Table 6. Immediately after electroporation, cells were placed in recovery media (RM) comprising EPSFM+LDL plus 5% pooled human umbilical cord blood plasma, and placed on ice for a further 10 minutes. Cells were then incubated at 37.degree. C. for 18 to 24 hours, after which samples were washed in wash buffer (WB), comprising PBS with 1% BSA and 0.1% sodium azide, pH 7.2). Transfection efficiency was measured using either the reporter plasmid pGREENLANTERN.TM. (which contains the gene coding for green fluorescent protein (GFP) from the Aequorea victoria jellyfish with a codon sequence "humanized" for efficient translation in human cells and transcribed from the CMV immediately early enhancer/promoter) or the pEGFP plasmid (which contains the GFP gene, human codon-optimized and containing a chromophore mutation which produces fluorescence 35 times more intense than wild-type GFP also transcribed from the CMV promoter). Cells were resuspended in 0.5 ml of WB and analyzed by flow cytometry for the expression of the GFP gene on a Coulter Epics Elite FACScan using forward and side scatter and fluorescence intensity in the fluorescein isothiocyanate (FITC) band pass (excitation at 488 nm/absorption at 525 nm). The transfection efficiency was determined as the percentage of GFP positive cells in transfected samples minus the percentage of positive cells in the mock transfected sample. Electroporated freshly isolated CD34.sup.+ cells were transfected to a maximal efficiency of 2.7% (see Table 6).

US-PAT-NO: 6344356

DOCUMENT-IDENTIFIER: US 6344356 B1

\*\*See image for Certificate of Correction\*\*

TITLE: Methods for recombining nucleic acids

DATE-ISSUED: February 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer, Willem P.C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 590778

DATE FILED: June 8, 2000

PARENT-CASE:

This application is a CON of Ser. No. 08/621,859 filed Mar. 25, 1996, now U.S. Pat. No. 6,117,679; which is a CIP of Ser. No. 08/564,955 filed Nov. 30, 1995, now U.S. Pat. No. 5,811,238; which is a CIP of Ser. No. 08/537,874 filed Mar. 4, 1996, now U.S. Pat. No. 5,830,721; which is the national phase of PCT/US95/02126 filed Feb. 17, 1995; which is a CIP of Ser. No. 08/198,431 filed Feb. 17, 1994, now U.S. Pat. No. 5,605,793; the disclosures of which are herein incorporated by reference.

US-CL-CURRENT: 435/440, 536/23.1 , 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

37 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotypic spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random,

pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed. semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernel set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (573):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

Detailed Description Text - DETX (589):

E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present



in the 'Clontech' construct, the expression vectors and GFP promoters are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6331527

DOCUMENT-IDENTIFIER: US 6331527 B1

\*\*See image for Certificate of Correction\*\*

TITLE: Promoter smooth muscle cell expression

DATE-ISSUED: December 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Parmacek; Michael S.	Bryn Mawr	PA	N/A	N/A
Solway; Julian	Glencoe	IL	N/A	N/A

APPL-NO: 09/ 431349

DATE FILED: November 1, 1999

PARENT-CASE:

This is a divisional of co-pending application Ser. No. 08/726,807, filed Oct. 7, 1996, which claims the benefit of priority of U.S. Provisional Application No. 60/004,868, filed Oct. 5, 1995.

US-CL-CURRENT: 514/44, 435/455, 435/456, 536/24.1, 623/1.13, 623/1.41

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

75 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX (82):

In order to identify the functionally important cis-acting sequences that regulate transcription of the SM22.alpha. gene in SMCs, a series of transient transfections were performed using SM22.alpha.-luciferase reporter constructs

and primary rat aortic vascular SMCs and the SMC line, A7r5, both of which express high levels of SM22.alpha. mRNA. Transfection of A7r5 cells with the plasmid p-5000/11SM22luc, containing 5-kb of 5' flanking sequence and the entire 4-kb SM22.alpha. intron 1 sequence (the initiation codon is located in exon 2), resulted in a 250-300-fold induction in luciferase activity as compared to the promoterless control plasmid, pGL2-Basic (FIG. 1A, lanes 1 and 2). This level of transcriptional activity was comparable to that obtained following transfection of A7r5 cells with the RSV-containing luciferase reporter plasmid, pRSVL (FIG. 1A, lanes 2 and 8). In order to determine whether this transcriptional activity was due to the immediate 5' flanking region of the SM22.alpha. gene, or alternatively, was due to a transcriptional regulatory element located within the first intron of the SM22.alpha. gene, the activities of the p-5000/11SM22luc and p-5000SM22luc plasmid were compared (FIG. 1A, lanes 2 and 3). Transfection of A7r5 cells with the p-5000SM22luc plasmid, containing only 5-kb of 5' flanking sequence, resulted in high-level transcription of the luciferase reporter gene comparable (on a molar basis) to levels obtained with the p-5000/11SM22luc plasmid. Thus, the 5' flanking region of the SM22.alpha. gene contains cis-acting sequence elements required for high-level transcription in A7r5 cells.

#### Detailed Description Text - DETX (148):

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the gene expression is down-regulated to non-detectable levels in "synthetic SMCs" located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative regulatory mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive regulatory factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the luciferase activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNAmyYY1 expression plasmid that encodes a mutant YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a luciferase reporter plasmid under the transcriptional control of the

SM22.alpha. promoter which has been mutagenized to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a negative regulatory factor. Conversely, the demonstration that over-expression of YY1 increases SM22.alpha. promoter activity would suggest (but not prove) that, as with the c-fos promoter, YY1 acts as a positive regulatory factor (Natesan and Gilman, 1995b).

US-PAT-NO: 6323030

DOCUMENT-IDENTIFIER: US 6323030 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: November 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer; Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 240310

DATE FILED: January 29, 1999

PARENT-CASE:

This application is a CON of Ser. No. 08/621,859 filed Mar. 25, 1996, now U.S. Pat. No. 6,117,679, which is a CIP of Ser. No. 08/564,955 filed Nov. 30, 1995, now U.S. Pat. No. 5,811,238, which is a CIP of Ser. No. 08/537,874 now U.S. Pat. No. 5,830,721, which is a 371 of PCT/US95/02126 filed Feb. 17, 1995, which is a CIP of Ser. No. 08/198,431 filed Feb. 17, 1994 now U.S. Pat. No. 5,605,793; the disclosures of which are hereby incorporated by reference.

US-CL-CURRENT: 435/440, 435/6 , 536/23.1 , 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

26 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (91):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (98):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (103):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random,

pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

#### Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR) Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

#### Detailed Description Text - DETX (535):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

#### Detailed Description Text - DETX (551):

E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present

in the 'Clontech' construct, the expression vectors and **GFP promoters** are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.



US-PAT-NO: 6297221

DOCUMENT-IDENTIFIER: US 6297221 B1

**\*\*See image for Certificate of Correction\*\***

TITLE: Method for promoting angiogenesis with a nucleic acid construct comprising an SM22.alpha.0 promoter

DATE-ISSUED: October 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Parmacek; Michael S.	Chicago	IL	N/A	N/A
Solway; Julian	Glencoe	IL	N/A	N/A

APPL-NO: 09/ 225670

DATE FILED: January 5, 1999

PARENT-CASE:

This is a divisional of application Ser. No. 08/726,807 filed Oct. 7, 1996, now U.S. Pat. No. 6,090,618, from U.S. Provisional Application No. 60,004,868, filed Oct. 5, 1995.

US-CL-CURRENT: 514/44

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

15 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX (98):

In order to identify the functionally important cis-acting sequences that regulate transcription of the SM22.alpha. gene in SMCs, a series of transient

transfections were performed using SM22.alpha.-luciferase reporter constructs and primary rat aortic vascular SMCs and the SMC line, A7r5, both of which express high levels of SM22.alpha. mRNA. Transfection of A7r5 cells with the plasmid p-5000/11SM22luc, containing 5-kb of 5' flanking sequence and the entire 4-kb SM22.alpha. intron 1 sequence (the initiation codon is located in exon 2), resulted in a 250-300-fold induction in luciferase activity as compared to the promoterless control plasmid, pGL2-Basic (FIG. 1A, lanes 1 and 2). This level of transcriptional activity was comparable to that obtained following transfection of A7r5 cells with the RSV-containing luciferase reporter plasmid, pRSVL (FIG. 1A, lanes 2 and 8). In order to determine whether this transcriptional activity was due to the immediate 5' flanking region of the SM22.alpha. gene, or alternatively, was due to a transcriptional regulatory element located within the first intron of the SM22.alpha. gene, the activities of the p-5000/11SM22luc and p-5000SM22luc plasmid were compared (FIG. 1A, lanes 2 and 3). Transfection of A7r5 cells with the p-5000SM22luc plasmid, containing only 5-kb of 5' flanking sequence, resulted in high-level transcription of the luciferase reporter gene comparable (on a molar basis) to levels obtained with the p-5000/11SM22luc plasmid. Thus, the 5' flanking region of the SM22.alpha. gene contains cis-acting sequence elements required for high-level transcription in A7r5 cells.

#### Detailed Description Text - DETX (164):

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the gene expression is down-regulated to non-detectable levels in "synthetic SMCs" located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative regulatory mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs ( see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive regulatory factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY 1 in C2C 12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the luciferase activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNA<sup>my</sup>YY1 expression plasmid that encodes a mutant YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a

luciferase reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been **mutagenized** to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a negative regulatory factor. Conversely, the demonstration that over-expression of YY1 increases SM22.alpha. promoter activity would suggest (but not prove) that, as with the c-fos promoter, YY1 acts as a positive regulatory factor (Natesan and Gilman, 1995b).

US-PAT-NO: 6291242

DOCUMENT-IDENTIFIER: US 6291242 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: September 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer, Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 165060

DATE FILED: October 2, 1998

PARENT-CASE:

This application is a continuation of Ser. No. 08/621,859, filed Mar. 25, 1996 now U.S. Pat. No. 6,117,679, which is a continuation-in-part of Ser. No. 08/564,955, filed Nov. 30, 1995 now U.S. Pat. No. 5,811,238, which is a continuation-in-part of Ser. No. 08/537,874, filed Mar. 4, 1996, now U.S. Pat. No. 5,830,721 which is the U.S. national phase of PCT/US95/02126, filed Feb. 17, 1995, which is a continuation-in-part of Ser. No. 08/198,431, filed Feb. 17, 1994, now U.S. Pat. No. 5,605,793 the disclosures of which are incorporated by reference.

US-CL-CURRENT: 435/440, 257/E29.242 , 257/E29.265 , 257/E29.313  
, 257/E39.016 , 435/6 , 435/91.2 , 536/23.1 , 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

21 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

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Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment

of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed **mutagenesis**. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR **mutagenesis** has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed **mutants** (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical **mutagenesis** (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv **mutants**. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region **sequences by randomizing the sequence in a synthetic** CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of **synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence** kernel set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by **mutating** the CDR(s) with site-directed **mutagenesis**, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (534):

A **synthetic gene** was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled **mutant** showed a 42-fold improvement of signal over the synthetic construct.

Detailed Description Text - DETX (551):

E. coli expressing the synthetic **GFP** construct ('wt') with altered **codon** usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and **GFP promoters** are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6291211

DOCUMENT-IDENTIFIER: US 6291211 B1

\*\*See image for Certificate of Correction\*\*

TITLE: Promoter for smooth muscle cell expression

DATE-ISSUED: September 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Parmacek; Michael S.	Chicago	IL	N/A	N/A
Solway; Julian	Glencoe	IL	N/A	N/A

APPL-NO: 09/ 431414

DATE FILED: November 1, 1999

PARENT-CASE:

This is a divisional of application Ser. No. 08/726,807, filed Oct. 7, 1996, now U.S. Pat. No. 6,090,618, which claims the benefit of priority to U.S. Provisional Application No. 60/004,868 filed Oct. 5, 1995.

US-CL-CURRENT: 435/69.1, 435/455, 435/456, 435/91.3, 514/44

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

32 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

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Detailed Description Text - DETX (107):

In order to identify the functionally important cis-acting sequences that regulate transcription of the SM22.alpha. gene in SMCs, a series of transient transfections were performed using SM22.alpha.-luciferase reporter constructs

and primary rat aortic vascular SMCs and the SMC line, A7r5, both of which express high levels of SM22.alpha. mRNA. Transfection of A7r5 cells with the plasmid p-5000/11SM22luc, containing 5-kb of 5' flanking sequence and the entire 4-kb SM22.alpha. intron 1 sequence (the initiation codon is located in exon 2), resulted in a 250-300-fold induction in luciferase activity as compared to the promoterless control plasmid, pGL2-Basic (FIG. 1A, lanes 1 and 2). This level of transcriptional activity was comparable to that obtained following transfection of A7r5 cells with the RSV-containing luciferase reporter plasmid, pRSVL (FIG. 1A, lanes 2 and 8). In order to determine whether this transcriptional activity was due to the immediate 5' flanking region of the SM22.alpha. gene, or alternatively, was due to a transcriptional regulatory element located within the first intron of the SM22.alpha. gene, the activities of the p-5000/11SM22luc and p-5000SM221luc plasmid were compared (FIG. 1A, lanes 2 and 3). Transfection of A7r5 cells with the p-5000SM22luc plasmid, containing only 5-kb of 5' flanking sequence, resulted in high-level transcription of the luciferase reporter gene comparable (on a molar basis) to levels obtained with the p-5000/11SM22luc plasmid. Thus, the 5' flanking region of the SM22.alpha. gene contains cis-acting sequence elements required for high-level transcription in A7r5 cells.

#### Detailed Description Text - DETX (173):

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the gene expression is down-regulated to non-detectable levels in "synthetic SMCs" located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative regulatory mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive regulatory factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the luciferase activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNAmyYY1 expression plasmid that encodes a mutant YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a luciferase reporter plasmid under the transcriptional control of the



SM22.alpha. promoter which has been mutagenized to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a negative regulatory factor. Conversely, the demonstration that over-expression of YY1 increases SM22.alpha. promoter activity would suggest (but not prove) that, as with the c-fos promoter, YY1 acts as a positive regulatory factor (Natesan and Gilman, 1995b).

US-PAT-NO: 6284743

DOCUMENT-IDENTIFIER: US 6284743 B1

\*\*See image for Certificate of Correction\*\*

TITLE: Method for modulating smooth muscle cell proliferation

DATE-ISSUED: September 4, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Parmacek; Michael S.	Chicago	IL	N/A	N/A
Solway; Julian	Glencoe	IL	N/A	N/A

APPL-NO: 09/ 546550

DATE FILED: April 10, 2000

PARENT-CASE:

This is a continuation of application Ser. No. 09/258,367, filed Feb. 26, 1999, now U.S. Pat. No. 6,114,311, which is a divisional of application Ser. No. 08/726,807 filed Oct. 7, 1996, now U.S. Pat. No. 6,090,618, which claims the benefit of priority to U.S. Provisional Application No. 60/004,868, filed Oct. 5, 1995.

US-CL-CURRENT: 514/44, 435/375 , 435/69.1

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

23 Claims, 14 Drawing figures

Exemplary Claim Number: 1,2

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX (72):

In order to identify the functionally important cis-acting sequences that

regulate transcription of the SM22.alpha. gene in SMCs, a series of transient transfections were performed using SM22.alpha.-luciferase reporter constructs and primary rat aortic vascular SMCs and the SMC line, A7r5, both of which express high levels of SM22.alpha. mRNA. Transfection of A7r5 cells with the plasmid p-5000/1SM221uc, containing 5-kb of 5' flanking sequence and the entire 4-kb SM22.alpha. intron 1 sequence (the initiation codon is located in exon 2), resulted in a 250-300-fold induction in luciferase activity as compared to the promoterless control plasmid, pGL2-Basic (FIG. 1A, lanes 1 and 2). This level of transcriptional activity was comparable to that obtained following transfection of A7r5 cells with the RSV-containing luciferase reporter plasmid, PRSVL (FIG. 1A, lanes 2 and 8). In order to determine whether this transcriptional activity was due to the immediate 5' flanking region of the SM22.alpha. gene, or alternatively, was due to a transcriptional regulatory element located within the first intron of the SM22.alpha. gene, the activities of the p-5000/1SM221uc and p-5000SM221uc plasmid were compared (FIG. 1A, lanes 2 and 3). Transfection of A7r5 cells with the p-5000SM221uc plasmid, containing only 5-kb of 5' flanking sequence, resulted in high-level transcription of the luciferase reporter gene comparable (on a molar basis) to levels obtained with the p-5000/1SM221uc plasmid. Thus, the 5' flanking region of the SM22.alpha. gene contains cis-acting sequence elements required for high-level transcription in A7r5 cells.

#### Detailed Description Text - DETX (138):

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the gene expression is down-regulated to non-detectable levels in "synthetic SMCs" located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative regulatory mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME4 binds both SRF (a positive regulatory factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CARG box sequences (similar to those present in SME4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p441SM22-luc reporter plasmid into primary rat aortic SMCs and the luciferase activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNAmYY1 expression plasmid that encodes a mutant YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on

binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a luciferase reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been mutagenized to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a negative regulatory factor. Conversely, the demonstration that over-expression of YY1 increases SM22.alpha. promoter activity would suggest (but not prove) that, as with the c-fos promoter, YY1 acts as a positive regulatory factor (Natesan and Gilman, 1995b).

US-PAT-NO: 6180406

DOCUMENT-IDENTIFIER: US 6180406 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: January 30, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer; Willem P.C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 099015

DATE FILED: June 17, 1998

PARENT-CASE:

This application is a divisional of 08/621,859 filed Mar. 25, 1996, the disclosure of which is incorporated by reference, which is a continuation-in-part of U.S. Ser. No. 08/564,955, filed Nov. 30, 1995, now U.S. Pat. No. 5,811,238, and a continuation-in-part of U.S. Ser. No. 08/537,874, filed Mar. 4, 1996, now U.S. Pat. No. 5,830,721 which is the national phase of PCT/U.S. Ser. No. 95/02126, filed Feb. 17, 1995, which is a continuation-in-part of U.S. Ser. No. 08/198,431, filed Feb. 17, 1994 now U.S. Pat. No. 5,605,793.

US-CL-CURRENT: 435/440, 435/6, 435/91.2, 536/23.1, 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

69 Claims, 37 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into *E. coli* and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin 1, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the

V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed **mutagenesis**. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR **mutagenesis** has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed **mutants** (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical **mutagenesis** (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv **mutants**. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region **sequences by randomizing the sequence in a synthetic** CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of **synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence** kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by **mutating** the CDR(s) with site-directed **mutagenesis**, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (546):

A **synthetic gene** was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled **mutant** showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the **mutant** protein(s) were soluble and active. The three amino acid **mutations** thus guide the **mutant** protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous **mutant** variants rapidly and efficiently.

Detailed Description Text - DETX (562):

E. coli expressing the synthetic **GFP** construct ('wt') with altered **codon** usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of

poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and GFP promoters are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.



US-PAT-NO: 6172188

DOCUMENT-IDENTIFIER: US 6172188 B1

TITLE: Fluorescent proteins

DATE-ISSUED: January 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thastrup; Ole	DK-3460 Biker.o	N/A	N/A	DK
Tullin; S.o slashed.ren	slashed.d	N/A	N/A	DK
Poulsen; Lars Kongsbak	DK-2860-S.o slashed.borg	N/A	N/A	DK
Bj.o slashed.rn; Sara	DK-2840 Holte	N/A	N/A	DK
Petersen	DK-2800 Lyngby			

APPL-NO: 08/ 819612

DATE FILED: March 17, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/DK96/00051 filed Jan. 31, 1996 , the contents of which is fully incorporated herein by reference, and claims priority of Danish application serial no. 1065/95 filed Sep. 22, 1995.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1065/95	September 22, 1995

US-CL-CURRENT: 530/350, 435/325 , 435/69.1 , 530/808

ABSTRACT:

The present invention relates to novel variants of the fluorescent protein GFP having improved fluorescence properties.

15 Claims, 12 Drawing figures

Exemplary Claim Number: 1,7,8

Number of Drawing Sheets: 12

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Detailed Description Text - DETX (33):

Restriction endonuclease sites inserted in the 5' (a HindIII site) and 3' (EcoRI and BamHI sites) primers facilitated the cloning of the PCR amplified **GFP** cDNA into a slightly modified pUC19 vector. The details of the construction are as follows: LacZ Shine-Dalgarno AGGA, immediately followed by the 5' HindIII site plus an extra T and the **GFP** ATG **codon**, giving the following DNA sequence at the lacZ-**promoter GFP** fusion point: P.sub.LacZ -AGGAAAGCTTTATG-**GFP**. At the 3' end of the **GFP** cDNA, the base pair corresponding to nucleotide 770 in the published **GFP** sequence (GenBank accession No. M62653) was fused to the EcoRI site of the pUC19 multiple cloning site (MCS) through a PCR generated BamHI, EcoRI linker region).

Detailed Description Text - DETX (45):

The S65T-GFP **mutation** was described by Heim et al (Nature vol.373 pp. 663-664, 1995). F64L-S65T-GFP was constructed as follows: An E.coli expression vector containing Y66H-GFP was digested with restriction enzymes NcoI and XbaI. The recognition sequence of NcoI is located at position 173 and the recognition sequence of XbaI is located at position 221 in the F64L-Y66H-GFP sequence listed below. The large NcoI-XbaI vector fragment was isolated and ligated with a **synthetic NcoI-XbaI DNA linker of the following sequence**:

US-PAT-NO: 6165793

DOCUMENT-IDENTIFIER: US 6165793 A

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: December 26, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer, Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 09/ 075511

DATE FILED: May 8, 1998

PARENT-CASE:

This application is a continuation of 08/621,859 filed Mar. 25, 1996, the disclosure of which is incorporated by reference.

US-CL-CURRENT: 435/440, 435/6 , 536/23.1 , 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

62 Claims, 37 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

----- KWIC -----

Detailed Description Text - DETX (90):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template

polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into *E. coli* and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (97):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (102):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (168):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques

14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (174):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (466):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

Detailed Description Text - DETX (482):

E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and GFP promoters are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.

US-PAT-NO: 6117679

DOCUMENT-IDENTIFIER: US 6117679 A

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: September 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer, Willem P. C.	Los Gatos	CA	N/A	N/A

APPL-NO: 08/ 621859

DATE FILED: March 25, 1996

PARENT-CASE:

This application is a CIP of Ser. No. 08/564,955 filed Nov. 30, 1995 now U.S. Pat. No. 5,811,238 which is a CIP of Ser. No. 08/537,874 filed May 4, 1996 now U.S. Pat. No. 5,830,721 which is a 371 of PCT/US95/02126 filed Feb. 17, 1995 which is a CIP of Ser. No. 08/198,431 filed Feb. 17, 1994 and now U.S. Pat. No. 5,605,793.

US-CL-CURRENT: 435/440, 435/6, 536/23.1, 536/24.3

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

35 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 37

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Detailed Description Text - DETX (91):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be

created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide

Detailed Description Text - DETX (99):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (104):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (172):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random, pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region

of a human tetanus toxoid-binding Fab.

Detailed Description Text - DETX (179):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

Detailed Description Text - DETX (580):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The three amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

Detailed Description Text - DETX (597):

E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the N-terminal extension present in the 'Clontech' construct, the expression vectors and GFP promoters are quite different. The cause of the improved fluorescence signal is not enhanced expression level, it is improved protein performance.



US-PAT-NO: 6114311

DOCUMENT-IDENTIFIER: US 6114311 A

**\*\*See image for Certificate of Correction\*\***

TITLE: Method for modulating smooth muscle cell proliferation

DATE-ISSUED: September 5, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Parmacek; Michael S.	Chicago	IL	N/A	N/A
Solway; Julian	Glencoe	IL	N/A	N/A

APPL-NO: 09/ 258367

DATE FILED: February 26, 1999

PARENT-CASE:

This is a divisional of co-pending application Ser. No. 08/726,807 filed Oct. 7, 1996.

US-CL-CURRENT: 514/44

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

18 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX (72):

In order to identify the functionally important cis-acting sequences that regulate transcription of the SM22.alpha. gene in SMCs, a series of transient transfections were performed using SM22.alpha.-luciferase reporter constructs and primary rat aortic vascular SMCs and the SMC line, A7r5, both of which

express high levels of SM22.alpha. mRNA. Transfection of A7r5 cells with the plasmid p-5000/11SM22luc, containing 5-kb of 5' flanking sequence and the entire 4-kb SM22.alpha. intron 1 sequence (the initiation codon is located in exon 2), resulted in a 250-300-fold induction in luciferase activity as compared to the promoterless control plasmid, pGL2-Basic (FIG. 1A, lanes 1 and 2). This level of transcriptional activity was comparable to that obtained following transfection of A7r5 cells with the RSV-containing luciferase reporter plasmid, pRSVL (FIG. 1A, lanes 2 and 8). In order to determine whether this transcriptional activity was due to the immediate 5' flanking region of the SM22.alpha. gene, or alternatively, was due to a transcriptional regulatory element located within the first intron of the SM22.alpha. gene, the activities of the p-5000/11SM22.alpha. and p-5000SM22luc plasmid were compared (FIG. 1A, lanes 2 and 3). Transfection of A7r5 cells with the p-5000SM22luc plasmid, containing only 5-kb of 5' flanking sequence, resulted in high-level transcription of the luciferase reporter gene comparable (on a molar basis) to levels obtained with the p-5000/11SM22luc plasmid. Thus, the 5' flanking region of the SM22.alpha. gene contains cis-acting sequence elements required for high-level transcription in A7r5 cells.

#### Detailed Description Text - DETX (143):

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the gene expression is down-regulated to non-detectable levels in "synthetic SMCs" located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative regulatory mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive regulatory factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al, 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the luciferase activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNA<sup>mut</sup>YY1 expression plasmid that encodes a mutant YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a luciferase reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been mutagenized to abolish YY1 binding

activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a

US-PAT-NO: 6090618

DOCUMENT-IDENTIFIER: US 6090618 A

\*\*See image for Certificate of Correction\*\*

TITLE: DNA constructs and viral vectors comprising a smooth muscle promoter

DATE-ISSUED: July 18, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Parmacek; Michael S.	Chicago	IL	N/A	N/A
Solway; Julian	Glencoe	IL	N/A	N/A

APPL-NO: 08/ 726807

DATE FILED: October 7, 1996

US-CL-CURRENT: 435/320.1, 536/23.1, 536/23.5, 536/24.1

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

62 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX (103):

In order to identify the functionally important cis-acting sequences that regulate transcription of the SM22.alpha. gene in SMCs, a series of transient transfections were performed using SM22.alpha.-luciferase reporter constructs and primary rat aortic vascular SMCs and the SMC line, A7r5, both of which express high levels of SM22.alpha. mRNA. Transfection of A7r5 cells with the plasmid p-5000/11SM22luc, containing 5-kb of 5' flanking sequence and the entire 4-kb SM22.alpha. intron 1 sequence (the initiation codon is located in exon 2), resulted in a 250-300-fold induction in luciferase activity as

compared to the promoterless control plasmid, pGL2-Basic (FIG. 1A, lanes 1 and 2). This level of transcriptional activity was comparable to that obtained following transfection of A7r5 cells with the RSV-containing **luciferase** reporter plasmid, pRSVL (FIG. 1A, lanes 2 and 8). In order to determine whether this transcriptional activity was due to the immediate 5' flanking region of the SM22.alpha. gene, or alternatively, was due to a transcriptional regulatory element located within the first intron of the SM22.alpha. gene, the activities of the p-5000/1SM22luc and p-5000SM22luc plasmid were compared (FIG. 1A, lanes 2 and 3). Transfection of A7r5 cells with the p-5000SM22luc plasmid, containing only 5-kb of 5' flanking sequence, resulted in high-level transcription of the **luciferase** reporter gene comparable (on a molar basis) to levels obtained with the p-5000/1SM22luc plasmid. Thus, the 5' flanking region of the SM22.alpha. gene contains cis-acting sequence elements required for high-level transcription in A7r5 cells.

#### Detailed Description Text - DETX (171):

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the **gene expression is down-regulated to non-detectable levels in "synthetic SMCs"** located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative regulatory mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive regulatory factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CARg box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the luciferase activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNA<sub>YY1</sub> expression plasmid that encodes a **mutant** YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a luciferase reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been **mutagenized** to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids

encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a

US-PAT-NO: 6084089

DOCUMENT-IDENTIFIER: US 6084089 A

TITLE: Cold-inducible promoter sequences

DATE-ISSUED: July 4, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mine; Toshiki	Iwata-gun	N/A	N/A	JP
Ohyama; Akio	Iwata-gun	N/A	N/A	JP
Hiyoshi; Toru	Iwata-gun	N/A	N/A	JP
Kasaoka; Keisuke	Iwata-gun	N/A	N/A	JP

APPL-NO: 08/ 894731

DATE FILED: October 27, 1997

PARENT-CASE:

This application is the national phase under 35 U.S.C. .sectn.371 of PCT International Application No. PCT/JP96/03822 which has an International filing date of Dec. 26, 1996 which designated the United States of America .

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	7-351825	December 27, 1995

PCT-DATA:

APPL-NO: PCT/JP96/03822  
DATE-FILED: December 26, 1996  
PUB-NO: WO97/24449  
PUB-DATE: Jul 10, 1997  
371-DATE: Oct 27, 1997  
102(E)-DATE:Oct 27, 1997

US-CL-CURRENT: 536/24.1, 435/6 , 536/23.6 , 536/24.3

ABSTRACT:

This invention discloses a novel cold-inducible promoter which induces gene expression at low temperatures in potato tubers but which is scarcely induced in organs other than tuber or at normal temperature, which induces gene expression for a long time not less than five months. The promoters of this invention are the DNA sequence having a nucleotide sequence from first to 3546th nucleotide in the nucleotide sequence shown in SEQ ID NO: 1, or a part thereof having a cold-inducible promoter activity, or a DNA sequence having the same nucleotide sequence as said DNA sequences except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted

or added, which DNA sequence has a cold-inducible promoter activity and the DNA sequence having a nucleotide sequence from first to 4120th nucleotide in the nucleotide sequence shown in SEQ ID NO: 2, or a part thereof having a cold-inducible promoter activity, or a DNA sequence having the same nucleotide sequence as the said DNA sequences except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

3 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX (8):

Site-specific mutagenesis may be carried out by, for example, using a synthetic oligonucleotide primer complementary to a single-stranded phage DNA except that the desired mutation as follows. That is, using the above-mentioned synthetic oligonucleotide as a primer, a complementary chain is produced by a phage, and host bacterial cells are transformed with the obtained double-stranded DNA. The culture of the transformed bacterial cells is plated on agar and plaques are formed from a single cell containing the phage. Theoretically, 50% of the new colonies contain the phage having a single-stranded chain carrying the mutation and remaining 50% of the colonies contain the phage having the original sequence. The obtained plaques are then subjected to hybridization with a kinase-treated synthetic probe at a temperature at which the probe is hybridized with the DNA having exactly the same sequence as the DNA having the desired mutation but not with the original DNA sequence that is not completely complementary with the probe. Then the plaques in which the hybridization was observed are picked up, cultured and the DNA is collected.

Detailed Description Text - DETX (72):

Genomic clone LCIP2-10 was digested with a restriction enzyme Asp718 (Boehringer) and Asp718 fragment containing about 200 bp upstream of the initiation ATG codon was introduced into a plasmid pUC19 to obtain a recombinant plasmid p210A8. Using this plasmid (p210A8) as a template and using M13 primer RV (Takara) and 210A primer (5'-GTTACTCTTAATTTTTC-3' (SEQ ID NO: 8)), PCR (94.degree. C. for 20 seconds, 55.degree. C. for 30 seconds, 72.degree. C. for 60 seconds, 25 cycles) was performed. The amplified product was subcloned into TA cloning vector (Invitrogen) to prepare a vector p210Pro(200) containing only about 200 bp upstream of the initiation ATG codon. From this vector, the fragment containing about 200 bp upstream of the initiation ATG codon was isolated by digestion with restriction enzymes HindIII and XhoI (Takara) and inserted into the HindIII, SalI site of pHSG399 (Takara) to obtain a plasmid pHSG210(200). This plasmid was digested with HindIII and BamHI (Takara) and the isolated fragment of about 200 bp upstream of the ATG



codon was inserted into the 5' upstream region of a vector (pLUC101) prepared by substituting the .beta.-glucuronidase gene of pBI101 vector (Clontech) with a luciferase gene (Science 234: 856-859, 1986) to obtain pLUC210(200). On the other hand, XbaI (Takara) fragment containing about 1000 bp upstream of the initiation ATG codon of the genomic clone LCIP2-10 was inserted into pUC18 vector to obtain a vector (p210X1) and a fragment of 800 bp (about -200 to -1000 bp, upstream of ATG codon) was isolated from this vector by digestion with restriction enzyme Asp718. This fragment was inserted into the Asp718 site of pLUC210(200) to obtain a vector pLUC210(1000) for transformation, which vector contained the promoter region of about 1 kb and a luciferase gene as a reporter. The vectors for transformation (pLUC101 and pLUC210(1000)) were introduced into a bacterium *Agrobacterium tumefaciens* LBA4404 by triparental mating (Plant Gene Manipulation Manual, Kodansha, 1990) and the resultant was used for transformation of plants.

US-PAT-NO: 5869035

DOCUMENT-IDENTIFIER: US 5869035 A

TITLE: Methods and compositions for inducing complement  
destruction of tissue

DATE-ISSUED: February 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Link, Jr.; Charles J.	Clive	IA	N/A	N/A
Levy; John P.	West Des Moines	IA	N/A	N/A

APPL-NO: 08/ 748344

DATE FILED: November 13, 1996

US-CL-CURRENT: 424/93.7, 424/277.1 , 424/93.21 , 435/320.1 , 514/44

ABSTRACT:

The invention discloses methods and compositions for killing tumor cells in animals. Through transfer techniques, cancer cells are engineered to express an epitope which is targeted by natural antibodies causing complement destruction of transformed tumor cells that is typically associated with hyperacute xenograft rejection.

14 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Drawing Description Text - DRTX (5):

FIG. 4 is a photograph depicting lymphocytes transduced by murine LNChRG retroviral vector expressing a humanized, red shifted GFP mutant gene. Lymphocytes were transduced by phosphate depletion method. The living cells were visualized with an FITC filter at 100.times. magnification

Detailed Description Text - DETX (122):

The second series of vectors that will be cloned will not contain G418 drug selectable markers (neor). Instead, these vectors will contain variants of the green fluorescent protein (GFP). We will employ for our initial studies a recently developed codon optimized, red shifted mutant GFP gene used in the

above experiments. Since this marker allows the detection in vitro and in vivo of gene transfer without the need for fixation, .alpha.(1,3)GT transduced lymphocytes can be selected by FACS or observed directly in frozen tissues sections. The retroviral plasmid pLdi.alpha.CG will be cloned by inserting the .alpha.(1,3)GT gene under control of the doxycycline inducible **promoter** (diP). See FIG. 5.

#### Detailed Description Text - DETX (123):

The diP expression cassette (kindly provided by Dr. Reeves, Mass. Gen Hospital) contains a **mutated** tetracycline repressor (mtetR) expressed from CMV immediate early promoter. The modified tetR gene contains amino acid substitutions that result in binding of doxycycline (DCN) and then activation of the tetracycline responsive element (TRE) promoter region. The TRE element is fused to a truncated CMV promoter. Binding to the TRE element by the doxycycline and mtetR protein complex results in the induction of high level gene expression. The .alpha.(1,3) GT gene will be cloned just downstream of this promoter. The final vector once integrated and expressed in target lymphocytes should exhibit induced expression of .alpha.(1,3)GT protein and subsequent presentation of .alpha.(1,3)galactosyl epitopes on the cell surface. The .alpha.(1,3)GT gene will be PCR amplified from the pL.alpha.SN vector to include Sac II and XbaI restriction sites at the 5' and 3' end of the gene's open reading frame respectively. This PCR product will be restriction digested with Sac II and Xba I and cloned into the Sac II and XbaI site of plasmid pTRE (Clontech Corp., Palo Alto, Calif.) to obtain plasmid pTRE.alpha.. Plasmid pTet-on containing the mtetR and VP16 fusion gene under control of the CMV promoter will be PCR amplified from the plasmid to contain flanking Xba I sites and then cloned into the corresponding site of plasmid pTRE.alpha.. Restriction analysis and sequencing will be done to obtain intact mtetR gene inserts in both the 5' to 3' and 3' to 5' orientation to obtain pT.alpha.di5 and pT.alpha.di3 respectively. The doxycycline inducible cassette will next be cloned into plasmid pLhRGSN (provided by R. Muldoon, HGTRI, IA). This plasmid contains the same red shifted, **humanized hRGFP gene** in the LXS retroviral backbone. The .alpha.(1,3)GT expression cassettes from both the pT.alpha.di5 and pT.alpha.di3 plasmid will be cloned downstream of the hRGFP gene at the available Xba I site and blunt end ligated to the 3' LTR sequence. The resulting plasmids pLGT.alpha.di5 and pLGT.alpha.di3 will have constitutive GFP expression from the Maloney virus LTR and doxycycline inducible expression of .alpha.(1,3)GT.

US-PAT-NO: 5811238

DOCUMENT-IDENTIFIER: US 5811238 A

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

DATE-ISSUED: September 22, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stemmer; Willem P. C.	Los Gatos	CA	N/A	N/A
Cramer; Andreas	Mountain View	CA	N/A	N/A

APPL-NO: 08/ 564955

DATE FILED: November 30, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. Ser. No. 08/198,431 filed 17 Feb. 1994 and of U.S. Ser. No. 08/537,874 filed 4 Mar. 1996, which is a national phase application of PCT/US95/02126 filed 17 Feb. 1995, and which claims priority to U.S. Ser. No. 08/198,431.

US-CL-CURRENT: 435/6, 435/440 , 435/91.2 , 435/91.5

ABSTRACT:

A method for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a method for the production of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a method of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

22 Claims, 22 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

----- KWIC -----

Detailed Description Text - DETX (89):

The initial small population of the specific nucleic acid sequences having mutations may be created by a number of different methods. Mutations may be created by error-prone PCR. Error-prone PCR uses low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence. Alternatively, mutations can be introduced into the template polynucleotide by oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, a short sequence of the polynucleotide is removed from the polynucleotide using restriction enzyme digestion and is replaced with a synthetic polynucleotide in which various bases have been altered from the original sequence. The polynucleotide sequence can also be altered by chemical mutagenesis. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other agents which are analogues of nucleotide precursors include nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. Generally, these agents are added to the PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used. Random mutagenesis of the polynucleotide sequence can also be achieved by irradiation with X-rays or ultraviolet light. Generally, plasmid DNA or DNA fragments so mutagenized are introduced into E. coli and propagated as a pool or library of mutant plasmids.

Detailed Description Text - DETX (96):

By using multiple primers in parallel, sequences in excess of 50 kb can be amplified. Whole genes and whole plasmids can be assembled in a single tube from synthetic oligonucleotides by parallel PCR. Sequences can be randomly mutagenized at various levels by random fragmentation and reassembly of the fragments by mutual priming. Site-specific mutations can be introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides. A particularly useful application of parallel PCR is called sexual PCR.

Detailed Description Text - DETX (101):

This approach of mixing two genes may be useful for the humanization of antibodies from murine hybridomas. The approach of mixing two genes or inserting mutant sequences into genes may be useful for any therapeutically used protein, for example, interleukin I, antibodies, tPA, growth hormone, etc. The approach may also be useful in any nucleic acid for example, promoters or introns or 3' untranslated region or 5' untranslated regions of genes to increase expression or alter specificity of expression of proteins. The approach may also be used to mutate ribozymes or aptamers.

Detailed Description Text - DETX (166):

Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species (idiotype spectrum). The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V.sub.H and V.sub.L cassettes which can be combined. Furthermore, the V.sub.H and V.sub.L cassettes can themselves be diversified, such as by random,

pseudorandom, or directed mutagenesis. Typically, V.sub.H and V.sub.L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. (1993) Biochemistry 32: 8848 showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants. Barbas et al. (1992) op.cit. attempted to circumvent the problem of limited repertoire sizes resulting from using biased variable region sequences by randomizing the sequence in a synthetic CDR region of a human tetanus toxoid-binding Fab.

#### Detailed Description Text - DETX (172):

For generating diverse variable segments, a collection of synthetic oligonucleotides encoding random, pseudorandom, or a defined sequence kernal set of peptide sequences can be inserted by ligation into a predetermined site (e.g., a CDR). Similarly, the sequence diversity of one or more CDRs of the single-chain antibody cassette(s) can be expanded by mutating the CDR(s) with site-directed mutagenesis, CDR-replacement, and the like. The resultant DNA molecules can be propagated in a host for cloning and amplification prior to shuffling, or can be used directly (i.e., may avoid loss of diversity which may occur upon propagation in a host cell) and the selected library members subsequently shuffled.

#### Detailed Description Text - DETX (401):

A synthetic gene was synthesized having improved codon usage and having a 2.8-fold improvement of the E. coli whole cell fluorescence signal compared to the industry standard GFP construct (Clontech, Palo Alto, Calif.). An additional 16-fold improvement was obtained from three cycles of sexual PCR and visual screening for the brightest E. coli colonies, for a 45-fold improvement over the standard construct. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled mutant showed a 42-fold improvement of signal over the synthetic construct. The expression level in E. coli was unaltered at about 75% of total protein. The emission and excitation maxima of the GFP were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein(s) were soluble and active. The four amino acid mutations thus guide the mutant protein into the native folding pathway rather than toward aggregation. The results show that DNA sequence shuffling (sexual PCR) can solve complex practical problems and generate advantageous mutant variants rapidly and efficiently.

#### Detailed Description Text - DETX (417):

E. coli expressing the synthetic GFP construct ('wt') with altered codon usage yielded a nearly 3-fold greater whole cell fluorescence signal than cells expressing the 'Clontech' construct (FIG. 15A). The comparison was performed at full induction and at equal OD.sub.600. In addition to the substitution of poor arginine codons in the 'wt' construct and the absence of the Alanine

residue from the 'Clontech' construct, the expression vectors and GFP promoters are quite different. Therefore, we cannot be certain about the cause of the improved fluorescence signal.